DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date NOV 12 1999

1133 '99 NOV 15 P3:18

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	L-Se-methylselenocysteine
Firm:	PharmaSe, Inc.
Date Received by FDA:	October 20, 1999
90-day Date:	February 17, 2000

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after February 17, 2000.

Robert J. Moore, Ph.D.

95S-0316

RPT 59

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

NOV 12 1999

Julian E. Spallholz, Ph.D.
President & CEO
PharmaSe, Inc.
3416 Knoxville Avenue
Lubbock, Texas 79413

Dear Dr. Spallholz:

This is in response to your letter to the Food and Drug Administration (FDA) dated October 6, 1999 (received on October 20, 1999), making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413 of the Federal Food, Drug, and Cosmetic Act (the Act)) and 21 CFR 190.6. Your letter notified FDA of your intent to market a dietary supplement containing L-Se-methylselenocysteine (SeMC), a substance you assert is a new dietary ingredient.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

Your submission contained information that you believe establishes that the new dietary ingredient SeMC, when used under the conditions recommended or suggested in the labeling of the dietary supplements, will reasonably be expected to be safe. The information in your submission does not meet the requirements of 21 CFR 190.6 (copy enclosed). The submission required under the Act must contain a description of the dietary supplement or dietary supplements that contains, among other things, the level of the new dietary ingredient in the dietary supplement and the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions

of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement (see 21 CFR 190.6(b)(3)). You may submit an amended notification that cures the defects described above. If you market your product without submitting an amended notification that meets the requirements of 21 CFR 190.6, or less than 75 days after submitting such a notification, your product is considered adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Please contact us if you have any questions concerning this matter.

Sincerely,

Lynn A. Larsen, Ph.D.
Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

Enclosure

6 1748

Office of Special Nutricuticals
HFS 450
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.
Washington, DC 20204

October 6, 1999

Dear Sir:

PharmaSe, Inc would like to introduce into the health food market a non-protein amino acid, L-Se-methylselenocysteine (SeMC), following the 75 day waiting period as provided by law. This seleno-amino acid is naturally synthesized and is found in a number of plants commonly consumed in the human diet. Garlic, onions, leeks and broccoli are known to synthesize most notably this seleno-amino acid. Since selenium is not known to be an essential trace nutrient by plants of any kind, the concentration of selenium generally and Se-methylselenocysteine specifically in plants is totally dependent upon the distribution and concentration of selenium in the soils from which the plants are harvested. It is likely that many other plant species, as well as yeast, synthesize L-Se-methylselenocysteine as has been shown for Astragalus.

The major human dietary sources of selenium are animal meats and poultry, as well as fish. A secondary source of human dietary selenium is cereal grains. Many animal feeds, cattle, swine and poultry are fortified with selenium and therefore animal foods, as well as seafoods are an excellent source of bioavalible selenium for humans. Cereal grains are also good sources of human dietary selenium, but because the selenium is not a requirement for plant growth, the selenium content of cereal grains is also reflective of the soil selenium content in which the plant is grown and harvested. A third form of selenium for humans is from dietary supplements. Selenium supplements for humans followed that of animals (begun in 1973) beginning about 1978. Dietary selenium, an essential trace nutrient, ingested by humans is metabolized and incorporated into a number of selenoproteins now numbering 13, most notably the selenoenzymes of the glutathionine peroxidase family. These selenoenzymes provide an antioxidant function in vivo of reducing metabolic hydrogen peroxide to water and organic hydroperoxides to alcohols.

The chemical forms of selenium consumed by humans from animal foods are L-selenocysteine and L-selenomethionine. The forms of selenium consumed in plant foods are L-selenomethionine followed by lesser amounts of L-Se-methylselenocysteine. Lesser amounts of other selenium species likely exist in foods. Dietary supplements of selenium for humans have included sodium selenite, sodium selenate, L-

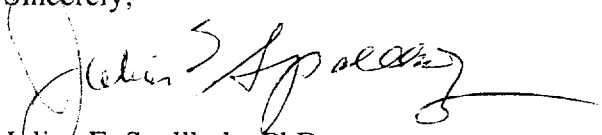
selenomethionine and a selenium containing yeast. These selenium supplements have been consumed for many years without any reports of human toxicity when ingested at levels of 200 ug selenium/day or less. A recent long term human study of 1312 persons with non-melanoma skin cancer were given 200 ug/Se/day of selenium yeast (mostly selenomethionine) for 4.5 years and revealed no toxicity and the epidemiological data suggested a reduction in lung, prostate and colorectal cancer in the selenium supplemented population. An even more recent report of humans consuming 200 ugSe/day reduced prostate cancer risk by one-third in 33,737 cohort members over seven years without adverse effects. The present Recommended Dietary Allowance (1989) for selenium is 70 ugSe/day for men and 55 ugSe/day for women.

The literature suggests and our own research shows that L-Se-methylselenocysteine has low toxicity relative to inorganic selenium compounds in animals and the toxicity of L-Se-methylselenocysteine is comparable L-selenomethionine toxicity. Tissue culture data reveals L-Se-methylselenocysteine toxicity to be far less toxic than inorganic selenium and again shows L-Se-methlyselenocysteine toxicity to be on a par with L-selenomethionine. The MSDS for L-Se-methylselenocysteine provides little toxicological information about the nutrient.

Dr. Clement Ip of Roswell Park Cancer Research Hospital will be or he may already have filed an IND with the FDA for the use of Se-methylselenocysteine in humans. Research plans are in place for eventual human research under a FDA approved IND.

We would appreciate any comments you may have on this natural selenoamino acid prior to its introduction into the health food industry.

Sincerely,



Julian E. Spallholz, PhD
President and CEO

PharmaSe.Inc.
3416 Knoxville Ave
Lubbock, TX 79413

Enclosures

Lessons from Basic Research in Selenium and Cancer Prevention[1,2]

Clement Ip

Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263

ABSTRACT The article reviews the progress in basic research of selenium and cancer prevention during the past decade. Special emphasis is placed on the following four major areas of discussion: *1*) chemical forms of selenium and anticarcinogenic activity; *2*) selenium-enriched food; *3*) in vitro effects of selenite vs. monomethylated selenium; and *4*) aromatic selenium compounds. It is clear that basic research has contributed new knowledge to our understanding of selenium biochemistry, anticancer efficacy and regulation of cell growth. Some of this information could be ready for incorporation into the design of a second-generation selenium trial in humans. J. Nutr. 128: 1845–1854, 1998.

KEY WORDS: • *selenium biochemistry* • *cancer prevention* • *animal models* • *cell growth regulation*

To researchers working in selenium and cancer prevention, the most exciting news in recent years is the finding by Clark et al. (1996) that supplementation of free-living people with selenized brewer's yeast was capable of decreasing the overall cancer morbidity and mortality by nearly 50%. The study was a double-blind, randomized, placebo-controlled trial involving 1312 patients (mostly men) who were recruited initially because of a history of basal cell or squamous cell carcinoma of the skin. Individuals in the treatment arm were given 200 μg Se/d for a mean of 4.5 y (average daily intake in the U.S. is about 100 μg). After a total follow-up of 8271 person-years, selenium treatment did not significantly affect the incidence of these non-melanoma skin lesions. However, patients receiving the Se-yeast supplement showed a much lower prevalence of developing and dying from lung, colon or prostate cancer. Statistical analyses verified that the relative risk of cancer incidence in lung, colon and prostate was reduced to 0.54 ($P = 0.04$), 0.37 ($P = 0.002$) and 0.42 ($P = 0.03$), respectively. Despite the fact that these are major cancers in the U.S. population, they could be considered only as secondary endpoints because the trial was originally set up to determine whether selenium would decrease the incidence of skin cancer.

A randomized, placebo-controlled intervention trial is the ultimate test to evaluate the efficacy of an anticancer agent. Before Clark's publication, there was already persuasive evidence in the literature suggesting a cancer protective effect of selenium in humans. Geographic correlation data in different regions worldwide and in the U.S. have long noted an inverse association between selenium levels in forage crops or diet and cancer mortality rates (Clark et al. 1991, Schrauzer et al. 1977, Shamberger et al. 1976, Yu et al. 1985). Several prospective and case-control studies also confirmed that people with low blood selenium had an increased risk of cancer (Clark et al. 1984 and 1993, Salonen et al. 1984 and 1985, Willett et al. 1983). Not all selenium and cancer epidemiology investigations produced uniform results because a handful of them failed to find an association (Coates et al. 1988, Knekt et al. 1988, Menkes et al. 1986, Nomura et al. 1987, Ringstad et al. 1988). The discrepancy is not unexpected because epidemiologic designs differ from one another and these diversities are frequently difficult to reconcile. Nonetheless, the potency of selenium is perhaps best exemplified by a meta-analysis of the combined data from a number of studies comparing the significance of serum selenium, retinol, β-carotene and vitamin E in relation to cancer risk (Comstock et al. 1992). Among these micronutrients, selenium emerged as the factor with the most consistent protective effect.

In view of the renewed interest in selenium and cancer, both in the scientific and lay communities, after the publication of Clark's project, it would be timely to examine what has been achieved in basic research during the past decade. The author has been an active participant in the field for many years. A patina of personal perspective is likely to permeate the article. This review is not intended to be all inclusive of every single paper published on the subject. Instead it will focus on four areas that may suggest the direction of our collective effort in the immediate future. In the introductory paragraph of a paper written by Howard Ganther more than 10 years ago (Ganther 1986), he stated that "it is important to keep in mind that the biological activity of selenium is an expression of selenium in a wide variety of chemical compounds, and not the element per se." This message is just as fitting now as ever and could in fact serve as the cornerstone of this review. Incidentally, Ganther has been a long-time collaborator and has contributed in many ways to much of the work in the author's laboratory.

[1]The work from the author's laboratory was supported by National Institutes of Health grants CA27706 and CA45164 (awarded to C.I.) and Institute Core grant CA16056.

[2]The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

Manuscript received 9 April 1998. Initial review completed 27 May 1998. Revision accepted 7 July 1998.

CHEMICAL FORMS OF SELENIUM AND ANTICARCINOGENIC ACTIVITY

One fascinating aspect of selenium biology is related to its extreme potency. Selenium, in the form of selenite or selenomethionine, functions as an essential micronutrient at levels of ~0.1 ppm (mg/kg) in the animal diet, but it becomes a toxin at levels of 8–10 ppm (Jacobs and Frost 1981). At the other extreme, selenium deficiency is customarily induced in laboratory animals by the feeding of a specially formulated diet which contains <0.01 ppm Se. It should be clarified at the outset that we will not deal with the effect of selenium deficiency on carcinogenesis. The information in this particular topic is not only sketchy but also inconsistent. For this reason, the review is limited to a discussion of the effect of selenium at levels above dietary requirement, usually in the range of 1–5 ppm Se. More than 90% of the selenium cancer chemoprevention experiments have used either sodium selenite or selenomethionine as the test reagent because they are commercially available. Both of these compounds are known to suppress carcinogenesis in many animal models (Combs 1997, El-Bayoumy 1991, Ip 1986, Medina and Morrison 1988). The effect is not organ specific, because tumor inhibition has been reported in mammary gland, liver, skin, pancreas, esophagus, colon and a few other sites. In general, there is a dose-dependent response, and selenium chemoprevention can be realized in the absence of toxicity.

On the basis of a large number of experiments that used a rat chemical-induced mammary tumor model, we showed that selenomethionine was not as active as selenite in cancer inhibition (Ip and Hayes 1989). Tissue selenium concentrations in blood, liver, kidney and skeletal muscle, on the other hand, were always higher in rats given selenomethionine compared with those given selenite. Therefore the greater total body burden of selenium in selenomethionine-treated rats did not appear to confer a better protection against tumorigenesis. The question that came to mind was whether selenium metabolism is necessary for its anticarcinogenic activity.

The above postulate was supported by additional indirect evidence from our laboratory. We found that a low methionine diet significantly reduced the protective effect of selenomethionine, even though tissue selenium was actually higher in these rats compared with those given an adequate amount of methionine (Ip 1988). When methionine is limiting, a greater percentage of selenomethionine is incorporated nonspecifically into body proteins in place of methionine (see **Fig. 1**) because met-tRNA cannot distinguish between methionine and selenomethionine. In other words, the anticarcinogenic activity of selenomethionine is severely compromised in a situation in which it is preferentially compartmentalized into tissue proteins instead of entering the metabolic pathway.

The schematic diagram in Figure 1 shows that methylation is a well-known fate of selenium metabolism (Ganther 1986). With a high intake of selenite or selenomethionine, the levels of methylated metabolites, including methylselenol, dimethyl selenide (expired in breath) and trimethylselenonium (excreted in urine), are expected to rise. Through the support of a collaborative research program with Ganther, we conducted a series of studies that were aimed at addressing the following questions: *1*) Does selenium have to flow through the intermediary inorganic hydrogen selenide pool for the cancer protective effect to be manifested? *2*) Does methylation of selenium enhance or diminish its chemopreventive efficacy? *3*) Is the degree of methylation important? Our strategy was to select precursor compounds that were capable of delivering selenium to specific locations along the methylation pathway

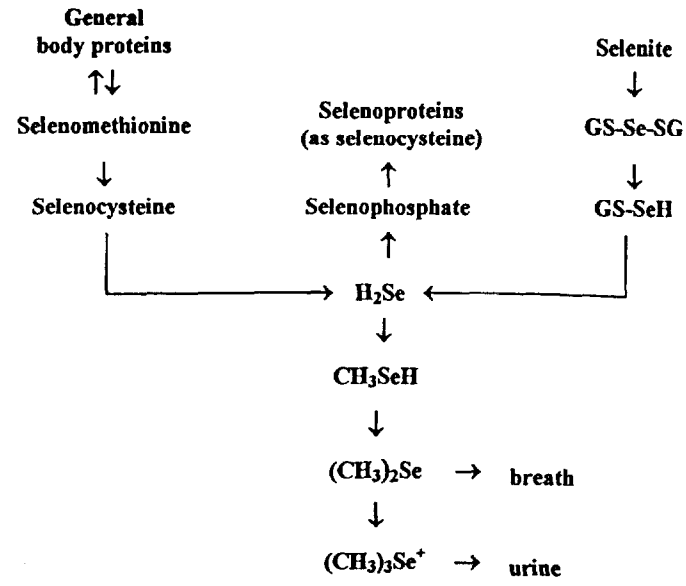


FIGURE 1 Selenium metabolic pathway. Selenomethionine can be incorporated into proteins in place of methionine because it readily acylates Met-tRNA. Alternatively it can be converted through the transsulfuration mechanism to selenocysteine, which in turn is degraded to hydrogen selenide (H_2Se) by the enzyme β-lyase. In contrast, selenite is metabolized to H_2Se via selenodiglutathione and glutathione selenopersulfide. Hydrogen selenide is generally regarded as the precursor for supplying selenium in an active form for the synthesis of selenoproteins. The further metabolism of H_2Se involves sequential methylation by S-adenosylmethionine to methylselenol, dimethylselenide and trimethylselenonium ion.

(Fig. 2). By this approach, we hoped to be able to pinpoint more closely the active intermediate that is involved in cancer protection (Ip and Ganther 1992). For a more detailed discussion of the biochemistry of selenium metabolism and the generation of potential chemopreventive metabolites, readers are urged to refer to a recent review by Ganther and Lawrence (1997).

Selenobetaine and Se-methylselenocysteine are good precursors for generating monomethylated selenium. As shown in Figure 2, selenobetaine tends to lose a methyl group first before scission of the Se-methylene carbon bond to form methylselenol (Foster et al. 1986a). Se-methylselenocysteine, on the other hand, is converted to methylselenol directly via a β-lyase reaction (Foster et al. 1986b), and unlike selenomethionine, it cannot be incorporated nonspecifically into proteins. We found that both selenobetaine and Se-methylselenocysteine were more efficacious than either selenite or selenomethionine in cancer chemoprevention in the range of 1–3 ppm Se (Ip and Ganther 1990 and 1992, Ip et al. 1991).

In contrast to the above two compounds, dimethylselenoxide undergoes rapid reduction to dimethylselenide. It had very low chemopreventive activity even at a level of 10 ppm Se (Ip et al. 1991). After a single oral dose of dimethylselenoxide, ~90% was recovered as exhalable dimethylselenide within a 24-h period (Vadhanavikit et al. 1993). Its facile conversion to dimethylselenide, which was then rapidly eliminated via the breath, could provide a plausible explanation for the low anticancer activity.

Selenobetaine methyl ester is known to undergo breakage of the Se-methylene carbon bond to form dimethylselenide directly (Foster et al. 1986a). However, the rate of conversion to dimethylselenide might not be as fast as that with dimethylselenoxide. Interestingly, the anticarcinogenic activity of

selenobetaine methyl ester was found to be comparable to that of selenobetaine (Ip and Ganther 1990). The metabolic profile studies also provided evidence that di- and trimethylated metabolites were capable of undergoing demethylation (Vadhanavikit et al. 1993). Because of the slower metabolism of selenobetaine methyl ester to dimethylselenide, some reverse traffic of dimethylselenide demethylation might occur, thereby attaining a critical level of methylselenol in this situation. The above explanation was supported by additional data indicating that there was considerably more back conversion to the inorganic H_2Se pool from selenobetaine methyl ester than from dimethylselenoxide (Ip and Ganther 1992).

In summary, our studies indicated that the formation of H_2Se is not essential for the expression of anticarcinogenic activity. Precursor selenium compounds that are able to produce a steady stream of monomethylated metabolite are likely to have good chemopreventive activity. On the other hand, selenium compounds that are rapidly metabolized to exhalable dimethylselenide are likely to be poor candidates. The degree of methylation is also an important factor. Our results showed that the fully methylated form, trimethylselenonium, was totally ineffective (Ip and Ganther 1988), probably because it was quantitatively excreted in urine (Vadhanavikit et al. 1993). The poor tissue retention of this compound might account for its low biological activity.

In an attempt to improve the anticarcinogenic activity of the monomethylated selenium derivative, we had also examined a series of aliphatic selenocyanates with increasing length of the carbon side chain, CH_3-$(CH_2)_n$-SeCN, in which $n = 0$, 2, 4 or 6. Selenocyanates (RSeCN) were used as the carrier of selenium because they are known to be efficiently metabolized to selenols (RSeH) and therefore represent a convenient precursor compound. Our bioassay data showed that the order of chemopreventive potency for these aliphatic selenocyanates was as follows: heptyl = pentyl > propyl > methyl (Ip et al. 1995). Thus it appeared that the longer alkyl chain homologs might be superior to methyl selenocyanate. This was a novel finding and could offer further clues to the design of more powerful anticancer selenium compounds.

Selenized yeast was the supplement given to people in Clark's study (Clark et al. 1996). Contrary to previous reports in which less sophisticated methods were used in determining that selenomethionine was the major constituent in yeast, recent analysis by a state-of-the-art technique of high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS)[3] demonstrated that selenomethionine accounted for no more than 20% of all selenium-containing materials (Bird et al. 1997). In addition to selenomethionine, the other compounds that had been identified included selenocystine, Se-methylselenocysteine and selenoethionine (representing ~20%). On top of that, there were several unidentified peaks that combined to represent 40–50% of the total. Thus the selenized yeast actually contains a cocktail of selenium in a variety of chemical forms. Among these, we have some understanding only of selenomethionine and Se-methylselenocysteine. At this time, there are no data regarding whether these different compounds exert distinctive effects on cell biology or how they might differentially affect the multistep process of carcinogenesis. Translational research generally involves the flow of applied learning from laboratories to clinics. In selenium cancer prevention, we have an unusual scenario in which a human trial ironically magnifies the paucity of knowledge in basic science.

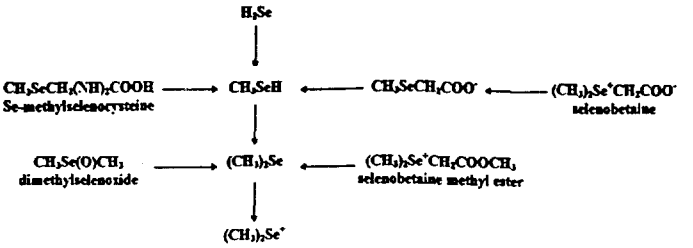


FIGURE 2 This schematic flow chart shows the main sites at which selenobetaine, Se-methylselenocysteine, selenobetaine methyl ester and dimethylselenoxide enter the selenium metabolic pathway below the H_2Se step.

RESEARCH ON SELENIUM-ENRICHED GARLIC

The intervention trial of Clark et al. (1996) is a classic example of "targeted chemoprevention" in which a particular substance is given to high risk individuals for the purpose of reducing cancer morbidity. There is a second concept of chemoprevention that is aimed at providing cancer protective chemicals to large segments of the population that are not at an increased risk because of known exposure to carcinogens, genetic predisposition or prior diagnosis of malignancy. Because of the intrinsic requirement of this plan for a wide distribution method, an expeditious way of delivering these protective agents is through the food system. Incidentally, a driving force for general population chemoprevention can be traced to the mounting epidemiologic and experimental data that strongly suggest the beneficial effects of various plant constituents present in our diet.

It is almost impossible to increase selenium intake by eating certain types of food because most common foods have a very low selenium content (Morris and Levander 1970). In the early 1990s, Ip and Lisk started a project in which they tried to enrich garlic with selenium by fertilizing the crop with water-soluble selenite salt. The idea was stimulated by the fact that plants are known to convert inorganic selenium in soil to organic selenium compounds following the sulfur assimilatory pathway (Shrift 1973). Because garlic contains an abundance of sulfur derivatives, it might be able to accumulate high levels of selenium. Initially, our goal was to see whether the idea could be put into practice and if so, to characterize the biological activities of this Se-garlic.

By controlling the intensity and frequency of selenite fertilization, Lisk was successful in cultivating Se-garlic enriched with a low of 100 ppm to a high of 1300 ppm Se dry weight. As a point of reference, natural garlic sold in the grocery stores contains <0.05 ppm Se. After harvest and processing, the Se-garlic was usually lyophilized and milled to a powder for feeding in animal research (Ip et al. 1992). We have published a series of papers with this material. Selected findings from these studies are summarized below.

A dose-dependent cancer protective effect was expressed in the range of 1–3 ppm Se in the diet (Ip and Lisk 1994a and 1994b). Total tumor yield was consistently reduced by 50–60% with 2 ppm Se supplementation. To ascertain that the efficacy of Se-garlic in cancer protection was primarily dependent on the action of selenium, we compared the effects of two batches of garlic powder with different levels of selenium enrichment, 112 vs. 1355 ppm Se dry weight. To achieve 2

[3]Abbreviations used: DMBA, dimethylbenz(a)anthracene; HPLC-ICP-MS, high performance liquid chromatography-inductively coupled plasma-mass spectrometry; IDP, intraductal proliferations; LD_{50}, lethal dose (the dose age that will cause 50% mortality); MNU, methylnitrosourea; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; UDP, uridine diphosphate.

ppm Se in the diet with these two batches of garlic powder, the amount needed was 1.8% for the 112 ppm Se-garlic vs. 0.15% for the 1355 ppm Se-garlic. In this way, we could vary the intake of garlic powder by more than 10-fold but keep the intake of total selenium constant. The results from several experiments led to the conclusion that the anticancer activity of Se-garlic was primarily accounted for by the effect of selenium, rather than the effect of garlic per se (Ip and Lisk 1995).

With the use of the rat dimethylbenz(a)anthracene (DMBA) model, we reported that supplementation of Se-garlic was capable of inhibiting both the initiation and postinitiation stages of mammary carcinogenesis (Ip and Lisk 1994b). DMBA is a procarcinogen requiring metabolic conversion to the ultimate carcinogen, DMBA-3,4-diol-1,2-epoxide, which then reacts with DNA to form adducts (Dipple et al. 1983, Liu and Milner 1992). Adduct formation is therefore the first manifestation of genotoxicity by the initiated cells. After absorption from the intestinal tract, DMBA undergoes first-pass metabolism in the liver. Although the liver is not a target site for DMBA-induced carcinogenesis, DMBA adducts are known to be present in liver DNA. After leaving the liver, some of the activated DMBA metabolites travel via the circulation to the mammary gland. Thus an analysis of DMBA adducts in both mammary cells and liver would provide confirmatory information of changes in DMBA metabolism. Our research showed that three types of adducts, *anti*-dG, *anti*-dA and *syn*-dA, were detected in mammary gland, whereas only the first two adducts were found in liver. Prior treatment with Se-garlic resulted in a consistent reduction of all DMBA-DNA adducts in both tissues (Ip and Lisk 1995 and 1997), suggesting that Se-garlic interfered with DMBA in causing genotoxic damage to DNA.

The decrease in DMBA adducts could be due to modulation of phase I and/or phase II xenobiotic metabolizing enzymes. Phase I enzymes are members of the cytochrome P450 system, which is responsible for converting chemical carcinogens to both electrophilic and nonelectrophilic products. The enzyme P450 1A1 is believed to play a key role in the formation of DMBA-3,4-diol-1,2-epoxide (Morrison et al. 1991). Thus a reduction in the activity of P450 1A1 would be expected to cause a decrease in adduct levels. Defenses against carcinogenic injury, on the other hand, are provided by phase II enzymes [such as glutathione-S-transferase and uridine diphosphate (UDP)-glucuronyltransferase], which are involved in the removal of metabolites through conjugation with glutathione or glucuronic acid (Talalay 1992). An increase in the activity of these phase II detoxifying enzymes could diminish the availability of DMBA metabolites in interacting with DNA.

In addition to 1A1, we also examined four other liver P450 enzymes (1A2, 2B1, 2E1 and 3A4) to determine if there might be a more general effect on the P450 family. No significant alteration was detected in any of these liver P450 enzymes in rats treated with Se-garlic at 1, 2 or 3 ppm Se (Ip and Lisk 1997). In contrast, glutathione-S-transferase and UDP-glucuronyltransferase were elevated to a maximum of 2- to 2.5-fold in liver and kidney in a dose-dependent manner (Ip and Lisk 1997). Our data therefore implied that an increased detoxification of carcinogen via the phase II conjugating enzymes might represent a mechanism of tumor suppression by Se-garlic.

The lack of an effect on P450 enzymes is actually desirable. For the development of novel approaches to cancer chemoprevention, it is generally prudent to avoid targeting the P450 enzymes because of the following considerations. A given agent may suppress a particular P450 enzyme, which is important in the activation of a certain class of carcinogens. However, the same agent may enhance other P450 enzymes that are critical in activating a different class of carcinogens. Such a double-edged sword effect is a major reason for steering away from agents that act by modulating phase I enzymes. Additionally, interference with P450 enzymes may compromise the capability of drug metabolism. This is not a trivial matter because humans frequently consume a variety of drugs to combat illnesses or diseases.

In an attempt to investigate the mechanism of tumor inhibition during the postinitiation phase, we varied the duration of Se-garlic treatment to either one of the following two protocols after carcinogen dosing: *1*) a continuous feeding of Se-garlic for 5 mo until termination or *2*) a 1-mo feeding of Se-garlic and a return to the control diet for the remaining 4 mo. The experiment was repeated in two mammary cancer models in which rats were given a single dose of either DMBA or methylnitrosourea (MNU). Unlike DMBA, MNU is a direct alkylating agent that does not require metabolic activation. Despite differences in their chemical reactivity, both carcinogens produce predominantly mammary tumors when given systemically to rodents. In both models, we found that short-term treatment with Se-garlic for 1 mo was just as effective in cancer prevention as the continuous 5-mo regimen (Ip et al. 1996), suggesting that Se-garlic might irreversibly suppress the clonal expansion of transformed cells in their early stage of development. Plasma and mammary tissue selenium levels essentially returned to basal values within a few weeks after withdrawal of Se-garlic supplementation. Thus the outcome of cancer protection by the short-term intervention regimen was not due to a slow turnover and thus a lingering presence of selenium in the target organ or in the circulation.

The pathobiology of chemical carcinogenesis in the rat mammary gland has been well delineated (Russo et al. 1982). There is a specific structure called the terminal end bud, which is the primary site for the induction of mammary carcinoma. Within 2–3 wk after carcinogen dosing, enlargement of the terminal end bud, characterized by a localized piling up of intraductal cells, is detectable in histological sections. These transformed cells continue to proliferate until they fill up the duct. This type of preneoplastic lesions, known as "intraductal proliferations" or IDP, is the precursor for the eventual development of palpable carcinomas. Se-garlic could conceivably inhibit or even eliminate these IDP, thereby reducing the number of premalignant lesions that are normally present in the early stage of mammary carcinogenesis. Preliminary studies from our laboratory indicated that the total number of IDP was reduced by 50% in the Se-garlic fed rats 6 wk after MNU treatment (unpublished). This observation reinforces our belief that the IDP are likely to be the target sites of selenium chemoprevention.

Further studies also showed that Se-garlic was superior to selenomethionine in terms of its anticarcinogenic efficacy (Ip and Lisk 1996). Unlike selenomethionine, which produced large increases in tissue selenium accumulation, Se-garlic caused only modest elevations (Ip and Lisk 1996). These attributes of Se-garlic became clear when Se-methylselenocysteine was identified as the major selenium-containing constituent in Se-garlic (Cai et al. 1995). The discovery was made through a collaboration between the laboratories of Peter Uden and Eric Block. Considering that the Se-methylselenocysteine research (discussed in the last section) was done before the inception of the Se-garlic project, everything came around in full circle, although the coincidence was rather fortuitous.

As a prototype "designer food" for general population che-

TABLE 1

In vitro effects of selenite and methylated forms of selenium[1]

Endpoints	Selenite	Methylselenocyanate or Se-methylselenocysteine
Cell morphology	Extensive cytoplasmic vacuolization, cell detachment	Normal
Membrane damage	Yes	No
Cell growth inhibition	++++	++
DNA synthesis inhibition	++++	++
Cell cycle block	S/G_2-M	G_1
DNA single strand breaks	++++	None
Cell death	Necrosis	Apoptosis
Gadd gene induction	Late	Early

[1] The above information is based on the data published in Jiang et al. (1993), Kaeck et al. (1997), Lu et al. (1994, 1995b and 1996) and Wilson et al. (1992).

moprevention, Se-garlic has many desirable characteristics. Because garlic is used primarily in flavoring food, there is less danger of overconsumption. At nutritional levels of selenium intake, Se-garlic provides bioavailable selenium for the maintenance of selenoenzymes (Ip and Lisk 1993). At higher levels, it has potent anticancer activity but does not cause excessive selenium accumulation because its predominant organoselenium compound, Se-methylselenocysteine, is rapidly metabolized to di- and trimethylated excretory products (Fig. 2). It induces phase II detoxifying enzymes, thereby facilitating the endogenous removal of xenobiotics. Most interesting of all, it appears to block the development of preneoplastic lesions. This mode of action is particularly suitable for reducing cancer morbidity in sporadic cases. Because Se-methylselenocysteine cannot be incorporated nonspecifically into proteins, the amount of total selenium decays quickly from various tissues upon discontinuation of Se-garlic feeding. The lack of a persistent retention in the body might alleviate the concern of selenosis in humans.

IN VITRO EFFECTS OF SELENITE AND METHYLATED FORMS OF SELENIUM

Although a spectrum of activities has been attributed to selenium in in vitro studies, this section will focus mainly on events that are associated with cell growth inhibition. During the 1980s, there were numerous reports showing that selenite, at concentrations in the micromole range, suppressed cell proliferation in culture and induced cytotoxicity as documented by the standard cell viability assays. This topic was reviewed previously (Ip and Medina 1987, Medina and Morrison 1988). At that time, selenite was the compound of choice because it was easily available from commercial sources. When the research was shifted to the methylated selenium compounds in the early 1990s, the laboratory of Henry Thompson began generating a body of information that supported the concept of distinctive cellular responses to specific chemical forms of selenium. The work of Thompson and co-workers resulted in a series of papers that were aimed primarily at comparing the in vitro activities of selenite with that of methylselenocyanate or Se-methylselenocysteine (Jiang et al. 1993, Kaeck et al. 1997, Lu et al. 1994, 1995b and 1996, Wilson et al. 1992).

Perhaps the best way to describe this collection of data from Thompson's laboratory is to summarize them in a table so that the differences can be easily highlighted (Table 1). This format is simple to follow although it may lose some subtlety due to generalizations. Suffice it to note that all of the experiments were not necessarily conducted with the same cell culture model; however, many of the observations were reproducible in more than one model. Another issue that needs clarification is the relative potency of the reagents. To produce the type of responses shown in Table 1, both selenite and the methylated selenium compounds were paired on an equimolar basis usually in the range of 1–10 μmol/L. It was possible to heighten the responses to the methylated selenium compounds, but only if their concentrations were raised 5- to 10-fold.

Selenite, when present at concentrations of 5–10 μmol/L in the media, caused extensive cytoplasmic vacuolization of cells as well as cell detachment from the culture dish. Cell membrane leakage was evident and the damage usually intensified as a function of time. The methylated selenium compounds, on the other hand, did not produce overt signs of cytotoxic effect. When cells were exposed to 10 μmol/L or even higher concentrations of methylselenocyanate or Se-methylselenocysteine, their morphology appeared normal and they remained anchored to the dish. Cell growth inhibition was invariably seen with selenite treatment in a dose-dependent manner. This was accompanied by decreases in DNA synthesis and a block in the cell cycle at the S/G_2-M phase. Treatment with Se-methylselenocysteine also resulted in a lower rate of cell growth and DNA synthesis, but the magnitude of inhibition was modest. In contrast, cell cycle progression was blocked at the G_1 phase. One of the signature genotoxic responses to selenite was a marked elevation in DNA single strand breaks that occurred within a few hours. Such an outcome was absent with exposure to the methylated selenium compounds. Cell death by necrosis or acute lysis was another hallmark of the selenite effect. After the initial wave of cell swelling and lysis, some visible signs of apoptosis were evident in the longer cultures. In contrast, both methylselenocyanate and Se-methylselenocysteine were known to induce cell death predominantly by apoptosis, an event that was characterized by distinctive morphological (e.g., cell blebbing or condensation of chromatin) and biochemical (nonrandom nucleosomal fragmentation or DNA laddering) changes. Thus it is clear that the chemical form of selenium is a very important factor in eliciting defined cellular responses in the in vitro system.

The proliferation of eukaryotic cells is controlled at specific stages of the cell cycle by cyclins and cyclin-dependent kinases (Sherr 1996, Weinberg 1995). There are two recent studies from Medina's laboratory describing a link between selenium and cell cycle proteins. In the first study, which involved the

use of an asynchronized mammary epithelial cell culture model (Sinha et al. 1996), it was found that Se-methylselenocysteine caused a 57% drop in cdk2 kinase activity and a 74% decrease in cyclin E-cdk2 content (therefore compatible with a G_1 arrest observed in this study as well as in the studies of Thompson), whereas selenite actually increased the cdk2 kinase activity by 47% without much appreciable change (10–20% decrease) in either of the cyclins D1, E or A bound to cdk2. The selenite results were incongruous with a S/G_2-M arrest, suggesting that the inhibition of cell growth by selenite might be associated with some nonspecific genotoxic effect unrelated to regulation of cell cycle proteins.

Thompson's studies (Table 1) and the first Sinha study (Sinha et al. 1996) of cell cycling disruption were done at a single time point in cells that were not synchronized, thus making it difficult to elucidate whether the cell cycle clock was stopped or delayed. Synchronized cells, on the other hand, are able to provide more precise information on the timing of the cell cycle clock with respect to other cellular events. With this in mind, Sinha and Medina (1997) repeated the experiments with cells that were released from growth factor deprivation by refeeding them with regular medium, a method commonly employed for synchronization. Parallel cultures were set up so that the cells could be sampled at different time points. [^{3}H]Thymidine incorporation into control cells peaked 16 h after refeeding. At this time point, 60% of cells had entered the S phase. Se-Methylselenocysteine, which was added to the medium 6 h after refeeding, inhibited [^{3}H]thymidine incorporation by ~50% and caused a significant delay in the S phase for almost 18 h. It also produced a concomitant 54% reduction in cdk2 kinase activity (confirming the finding of the previous study). A decrease in cdk2 kinase would be expected to impede progress through the S phase. The level of cyclin E associated with cdk2 did show a transient decrease at an early time point, but it recovered, thereby allowing cells to cross the G_1/S boundary (recall the persistent decrease in cyclin E-cdk2 in asynchronized cells). In summary, the data demonstrated that inhibition of cell growth by Se-methylselenocysteine was due to a prolonged delay in the S phase that was coincident with a marked decrease in cdk2 kinase activity.

Inhibition of cell growth can be accomplished by either a decrease in cell proliferation or an increase in apoptosis or both. Apoptosis is therefore an important cellular mechanism for growth regulation. Despite the conclusion from Thompson's work that selenite preferentially causes necrotic cell death, other reports have suggested otherwise. Recently, Stewart et al. (1997) tried to quantitate the proportion of apoptotic cells by the Apoptag method in a human colon cancer cell line treated with 10 μmol/L selenite. After 4 d, they found that as many as 40% of the cells were stained positive with the use of this assay, which is based on immunohistochemical detection of digoxigenin-labeled nucleotides added to the free 3′-hydroxyl ends generated as a result of DNA breaks. Because 5–10 μmol/L selenite is known to produce massive DNA strand breaks independently of apoptosis, the results of this study are difficult to interpret. Selenodiglutathione, a metabolite of selenite (Fig. 1), has also been examined by a different group of investigators. Lanfear et al. (1994) showed that selenodiglutathione was able to induce apoptosis as determined by fluorescence dye DNA-binding analysis. The principle of the assay is based on the discrimination that apoptotic cells will bind only the Hoechst 33342 dye, whereas necrotic cells will bind both the Hoechst dye and propidium iodide. Live cells do not bind either dye and therefore do not fluoresce. The different subpopulations can be sorted by flow cytometry based on their blue (Hoechst) or red (propidium iodide) fluorescence signals.

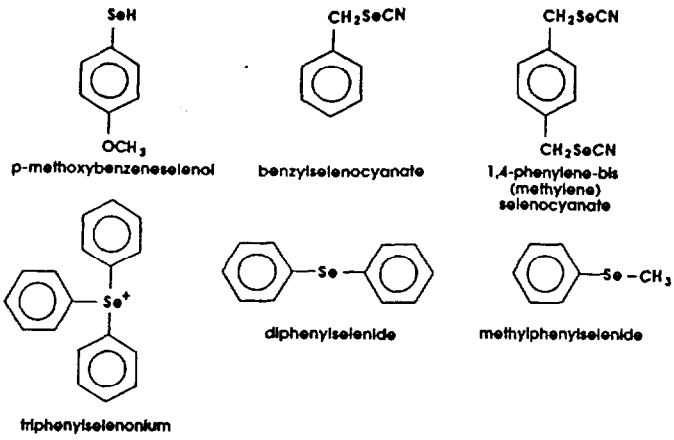


FIGURE 3 Structures of aromatic selenium compounds.

A careful examination of Lanfear's study revealed some rather curious findings in that the control culture (i.e., not treated with selenium) contained a large fraction of necrotic cells. The investigators never explained the presence of all these necrotic cells 6 h after plating when the culture should be in log growth. Upon incubating the culture with 3 μmol/L of selenodiglutathione, a small subset of apoptotic cells emerged in addition to an apparent increase in the number of necrotic cells. From the paper, it was difficult to tease out the results of percentage distribution of live cells, necrotic cells and apoptotic cells because no quantitative data were available. Nonetheless, the appearance of apoptotic cells was unmistakable because these blue fluorescent sorted cells also exhibited the typical DNA laddering pattern on gel electrophoresis.

There was one other piece of information tucked away in the paper that was of special interest. The experiment of Lanfear was done using mouse erythroleukemia cells, which are known to carry a p53 mutated gene, suggesting that a functional p53 pathway was not essential for selenium induction of apoptosis in these cells. The dissociation between wild-type p53 and apoptosis has since been described for the effect of methylselenocyanate in a mouse MOD mammary tumor cell subline with a null p53 phenotype (Kaeck et al. 1997) and for the effect of selenomethionine in HT29 colon cancer cells, which express a mutated p53 (Redman et al. 1997). Given that mutations in p53 are among the most common pathogenetic alterations in human cancers (Greenblatt 1994), an intervention mechanism based on the induction of apoptosis could provide a strong rationale for selenium chemoprevention in the human population. Further research should be focused on testing this hypothesis in vivo and on developing appropriate biomarkers associated with the control of apoptosis.

AROMATIC SELENIUM COMPOUNDS

Karam El-Bayoumy was the first to pioneer the research of aromatic selenium compounds in cancer chemoprevention in the 1980s. His idea originated from the need to develop novel reagents with a lower toxicity than that of selenite and selenomethionine. The chronology started with *p*-methoxybenzeneselenol (Fig. 3). In collaboration with other investigators at the American Health Foundation, El-Bayoumy reported successful tumor inhibition at different sites (liver, colon and kidney) by the feeding of 50 ppm of *p*-methoxybenzeneselenol (equivalent to

~20 ppm Se) to rats that were treated with the carcinogen azoxymethane (Reddy et al. 1985, Tanaka et al. 1985). This compound, however, was quickly abandoned in favor of benzylselenocyanate (Fig. 3), even though benzylselenocyanate was apparently more toxic. The dosage that causes 50% mortality (LD_{50}) of *p*-methoxybenzeneselenol and benzylselenocyanate in mice was 370 and 18 mg/kg body weight, respectively (El-Bayoumy 1985). Subsquent studies with benzylselenocyanate (El-Bayoumy 1985, Nayini et al. 1989 and 1991) showed that it suppressed tumorigenesis in several models including forestomach (benzo[a]pyrene), colon (azoxymethane) and mammary gland (DMBA). The carcinogen responsible for inducing cancer at each site is denoted parenthetically. In the above experiments, benzylselenocyanate was given in the diet at a concentration of 25 ppm (equivalent to 10 ppm Se); the schedule generally encompassed a relatively short time period, which started 2 wk before to 1 wk after carcinogen administration. The sulfur analog, benzylthiocyanate, was not effective, suggesting that there was specificity to selenium chemoprevention. The fact that benzylselenocyanate is able to block tumor induction by a variety of carcinogens at the initiation stage is intriguing because different P450 families are involved in the activation of benzo[a]pyrene, azoxymethane and DMBA. In the case of azoxymethane, Fiala et al. (1991) found that benzylselenocyanate increased its oxidative metabolism in the liver, thus resulting in a reduced delivery of methylazoxymethanol to the colon via the bloodstream. Consequently, there was less DNA alkylation in the colon, which was reflected by a diminished formation of O^6-methylguanine and 7-methylguanine. As far as the author is aware, the effect of benzylselenocyanate on polycyclic hydrocarbon metabolism has not been investigated.

Despite the initial intention to develop a less toxic compound, benzylselenocyanate actually fell short of this goal because at a level of 25 ppm in the diet, the rats suffered significant growth depression. Because benzylselenocyanate has a very strong odor similar to that of burnt rubber, the reduced food intake of animals noted in these experiments could be due to unpalatability of the diet. To reduce the volatility of benzylselenocyanate, a second methyleneselenocyanate group was added in the *para-* position to form 1,4-phenylenebis(methylene)selenocyanate (Fig. 3). This compound was commonly called *p*-xylylselenocyanate or *p*XSC. Acute LD_{50} and subchronic studies showed that *p*XSC was markedly less toxic than benzylselenocyanate (Conaway et al. 1992). A level of 80 ppm of *p*XSC (equivalent to 40 ppm Se) inhibited DMBA-induced mammary carcinogenesis in the initiation stage by suppressing the formation of DMBA-DNA adducts (El-Bayoumy et al. 1992). Whether this was due to modulation of P450 enzymes or phase II detoxifying enzymes remains to be determined. The anti-initiation effect was similarly observed in the azoxymethane-induced colon cancer model (Reddy et al. 1992). Additionally, *p*XSC also inhibited mammary and colon carcinogenesis in the postinitiation or tumor promotion phase (Ip et al. 1994a, Reddy et al. 1992), suggesting that it may have multiple mechanisms of action. Interestingly, prostaglandin E_2 was marginally decreased, whereas glutathione peroxidase was significantly increased in the colon of *p*XSC-treated rats. The significance of these findings with respect to cancer chemoprevention is unclear at the present time.

Some uniqueness of *p*XSC was highlighted in a NNK lung cancer chemoprevention experiment in mice (El-Bayoumy et al. 1993). NNK, which stands for 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, is a tobacco-specific carcinogen. *p*XSC at levels of 5, 10 and 15 ppm Se significantly reduced lung tumor multiplicity from 7.6 per mouse in the control group to 4.1, 3.3 and 1.8 per mouse, respectively. In contrast, selenite at 5 ppm Se had no protective effect. Consistent with the findings of these bioassays were the observations that *p*XSC decreased NNK-induced O^6-methylguanine formation in lung DNA, whereas selenite failed to produce a similar response (Prokopczyk et al. 1996). In rodents, α-hydroxylation of NNK is a major pathway of NNK metabolism (Hecht 1994). This key reaction leads to the formation of electrophiles, which can readily methylate and pyridyloxobutylate various macromolecules. The bioactivation of NNK is catalyzed by multiple P450 enzymes including 1A1, 2A1, 2B1, 2B2 and others that have not been characterized. In view of the fact that NNK is strongly implicated in the pathogenesis of tobacco-related lung cancer in humans (Hecht and Hoffmann 1988), it is important to elucidate the biochemical mechanisms by which *p*XSC modulates NNK metabolism as well as that of other nitrosoamines.

Attempts have also been made to compare *p*XSC with the closely related structural isomers *o*-XSC and *m*-XSC (*o* = ortho; *m* = meta) in the colon carcinogenesis model. Using aberrant crypt foci as the endpoint, all three compounds expressed comparable inhibitory effects: 47% for *o*-XSC, 49% for *m*-XSC and 66% for *p*-XSC (Reddy et al. 1994). Although the difference in biological activity was small, the isomers were not necessarily absorbed to the same extent by the intestinal tract. After an oral gavage, the percentage dose recovered in the feces in 2 d for *o*-XSC, *m*-XSC and *p*-XSC was 25, 60 and 75%, respectively (Sohn et al. 1995). The pharmacokinetics of these compounds in relation to their potency will have to be investigated more thoroughly.

With the benzyl-type selenium compound such as *p*XSC, some selenium is released from the parent molecule into the inorganic selenide pool. This possibility is supported by the evidence of nutritional bioavailability of selenium from *p*XSC as reported by Ip et al. (1994a). However, the rate of selenium release cannot explain entirely the anticarcinogenic activity of *p*XSC. The study of Ip et al. (1994a) showed that 10 ppm Se as *p*XSC was equivalent to 3 ppm Se as selenite in the efficacy of cancer protection. On the other hand, it took 1 ppm Se as *p*XSC to fully replete glutathione peroxidase in a selenium-deficient animal as opposed to only 0.1 ppm Se as selenite. Therefore, the ratio of anticancer activity to nutritional activity for *p*XSC is 10, as opposed to a ratio of 30 for selenite, suggesting that *p*XSC has certain inherent activity that is independent of the release of selenium from the parent molecule.

Compounds with selenium bonded directly to a benzene ring are very stable. There are no mammalian enzymes known that will catalyze the transfer of the benzene ring. For this reason, we decided to examine three phenyl selenide derivatives: triphenylselenonium, diphenylselenide and methylphenyl selenide (Fig. 3). Although they are related to each other structurally, they differ substantially in their chemical properties. Triphenylselenonium is positively charged and amphiphilic, whereas diphenyl selenide and methylphenyl selenide are uncharged and lipophilic.

Triphenylselenonium was a very effective chemopreventive agent in the experimental mammary cancer models (Ip et al. 1994b). At a level of 30 ppm Se supplemented in the diet, total tumor yield was suppressed by 60–70% in rats that had been treated with a mammary carcinogen. This dose level produced hardly any accumulation of total selenium in tissues, even under a chronic treatment condition. Preliminary studies indicated that it was very well tolerated by laboratory animals. No evidence of adverse symptoms was detected at levels up to 200 ppm Se. There is thus a wide margin separating the

chemopreventive dose range and the toxic dose range. Given the cationic and bulky nature of the molecule, the high tolerance is likely due to a poor rate of absorption via the enteral route. Fecal excretion after a single oral administration of triphenylselenonium was ~78 and 8% of the dose during d 1 and 2, respectively, suggesting that a large proportion of the gavage passed through the intestinal tract with minimal recirculation (Ip et al. 1997). Considering that so little is in fact taken up by the body, the in vivo activity of triphenylselenonium is truly fascinating.

The in vitro effect of triphenylselenonium was characterized mainly by cytostasis, i.e., a decrease in cell proliferation (due to inhibition of DNA synthesis) that was not accompanied by apoptotic cell death (Lu et al. 1995a). An agent that does not induce apoptosis will not be expected to cause deletion of transformed cells. Unless it is available continuously, the ability to protect against cancer would be lost when treatment is interrupted. This is the type of response predicted for triphenylselenonium. When triphenylselenonium was given continuously during the entire period of tumor promotion/progression (a 5-mo protocol), it was very effective in suppressing the development of tumors. However, when the treatment period was shortened to 1 mo after carcinogen dosing, there was a marked decrease in efficacy (Ip et al. 1998). At this point, it might be worthwhile to recall the data with Se-garlic in which a 1-mo treatment schedule was just as effective as the 5-mo schedule in cancer protection. As discussed in the previous section, the monomethylated selenium is a potent inducer of apoptosis. The elimination of early transformed preneoplastic cells might explain the outcome of sustaining a lower cancer risk even if treatment is discontinued after a short period of exposure to the anticancer agent.

In contrast to the high tolerance with triphenylselenonium, a significant drop in tolerance to no more than 30 ppm Se was noted with diphenylselenide (Ip et al. 1997). At this dose level, diphenylselenide was at best only half as active as triphenylselenonium in tumor inhibition. For diphenylselenide, fecal recovery was ~6 and 30% of the dose during d 1 and 2, respectively, and ~20% of the dose was recovered in the urine on each of the 2 d. The excretion profile suggested that most of the diphenylselenide dose was absorbed and that urinary excretion was a major route of elimination for diphenylselenide once it was absorbed. Even though diphenylselenide caused a two- to threefold increase in tissue selenium, it was less active than triphenylselenonium in cancer protection. The above experiments bring home the message that small changes in the structure of selenium compounds could lead to rather surprising changes in biological activity.

The surprises continued with methylphenyl selenide. Among the three phenylselenide derivatives, it was the least tolerated. A level of 5 ppm Se of methylphenyl selenide in the diet was the maximum that would produce no decreases in growth. On the basis of dose-response data in chemoprevention bioassays, methylphenyl selenide and Se-methylselenocysteine behaved quite similarly, although their structures are very different from each other. According to our results, the ED_{50} for methylphenyl selenide, triphenylselenonium and diphenylselenide was estimated to be ~2, 20 and >30 ppm Se, respectively. However, when measured against the scale of tolerance, triphenylselenonium was the best at >200 ppm Se and methylphenyl selenide the worst at 5 ppm Se. It is clear that as a class, the aromatic selenium compounds lag far behind the selenoamino acids on our learning curve. We know virtually nothing about their metabolism, pharmacology and toxicology. From what little has been discovered on the basic research side, their biochemistry is certainly very interesting. As of now, we simply do not have sufficient information to determine whether these aromatic selenium compounds and the selenoamino acids are acting via different mechanisms in chemoprevention.

CONCLUSION

The Clark study (Clark et al. 1996) was started in 1984. At that time, very little was known about the mechanism of action of selenium in cancer prevention. Fourteen years later, the gap has been narrowed but there is still a glaring void in our understanding of how selenium might block the clonal expansion of early malignant cells, especially at the molecular level. The science of cancer chemotherapy has long recognized the need to develop a close interaction among chemists, biochemists, pharmacologists, oncologists, pathologists, toxicologists, cell biologists and molecular biologists. Such a concerted enterprise is sorely lacking in the cancer chemoprevention arena. Currently there are hundreds of chemicals that have been and are being evaluated for anticancer activities in both in vivo and in vitro models. The cumulative effort is substantial, but there is little to demonstrate because the effort is so fragmented. Unless the community as a whole (including both commercial and public sectors) is willing to prioritize and commit the necessary resources for targeted research, the work on these hundreds of chemicals will proceed at the same agonizingly slow pace as we cross into the 21st century.

Of all the human cancer intervention studies that have been completed to date, the selenium trial is by far the most successful. The Clark study has probably attracted its share of skeptics because to put it bluntly, many may consider the results too good to be true. Therefore it needs to be repeated and it should be repeated with an improved design. During the last decade, the basic research side has contributed new knowledge of the relationship linking selenium biochemistry, anticarcinogenic potency and regulation of cell growth. Much of this information is on the verge of being ready for incorporation into a second-generation trial. The modulation of cell cycle proteins and apoptotic proteins by selenium is an emerging area of interest. Normal cells, early transformed cells and late stage preneoplastic cells may respond differently to selenium intervention with respect to these molecular pathways. The sooner we understand the fundamental mechanism of selenium chemoprevention, the closer we will be in finding a viable strategy in reducing cancer morbidity in the human population.

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Chemical Transformations of Selenium in Living Organisms. Improved Forms of Selenium for Cancer Prevention

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Abstract: Compounds having cancer-preventing activity are developed during the metabolism of selenium in plants and animals. Monomethylated forms of selenium appear to be one class of chemopreventive metabolites. Synthetic organoselenium compounds have been used to explore determinants of activity and differentiation from other biological effects of selenium. Triphenyl selenonium chloride, a new type of chemopreventive selenium compound, has been synthesized in radioactive form for use as a tracer to facilitate studies of its mode of action.

INTRODUCTION

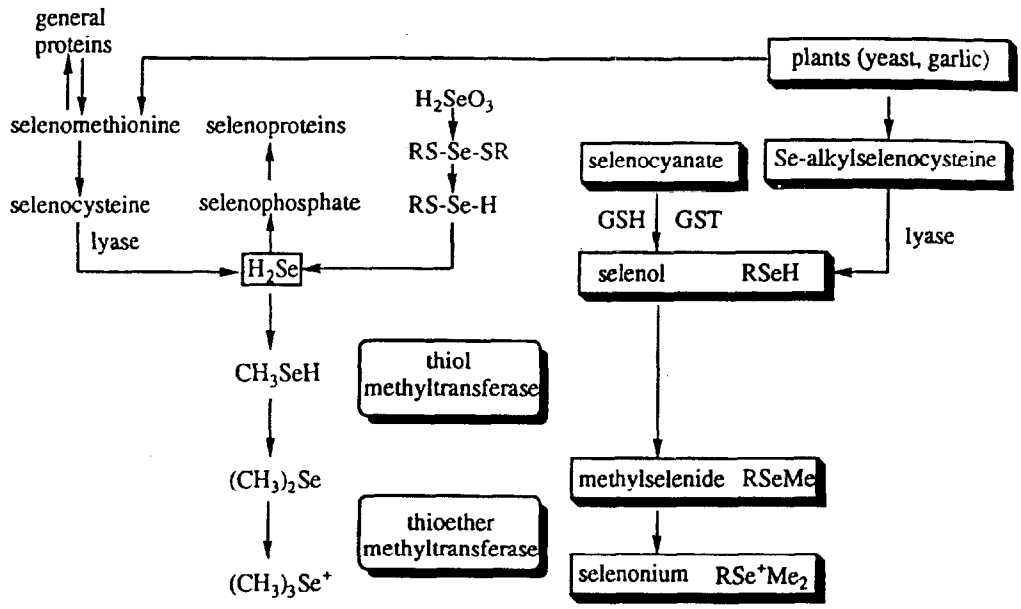
Selenium has been shown to prevent cancer in studies with experimental animals[1] and with humans[2]. The objectives of this article are to provide a perspective on the chemical fate of selenium in living organisms, and the origins of chemopreventive compounds when selenium is metabolized in plants and animals.

In animals, the activities of selenium as an essential nutrient, cancer-preventing agent, and toxicant, are developed as the dietary selenium level is increased over an approximately 100-fold range. As a point of reference, the nutritional requirement for selenium in animals is comparable to that for iodine, and the toxicity of inorganic sodium selenite is comparable to that of sodium arsenite. The nutritional requirement for selenium, like iodine, can be met by providing simple inorganic salts, and both selenium and iodine are metabolized in animals to their active organic forms.

Following the discovery in 1957 that selenium was an essential trace element for animals, considerable effort was made by Schwarz and others to isolate and identify a low molecular weight form of selenium ("Factor 3") that would be the putative active form[3]. Hundreds of organoselenium compounds were synthesized and fed to animals for asssay of biological activity in the prevention of selenium deficiency. These studies constitute a rich source of information on the relative bioavailability of selenium in different chemical forms, reflecting the ease with which selenium can be released from diverse chemical structures. However, this approach failed to identify any selenium compound that was more than a few-fold more active than inorganic selenium salts. Beginning with the discovery that selenium was an essential component of glutathione peroxidase[4], all the known functions of selenium as an essential nutrient in animals and certain microorganisms have been associated with selenoproteins. Usually these selenoproteins contain selenocysteine at the active site of an enzyme. There are elaborate mechanisms to ensure the specific incorporation of selenium into selenoproteins; assimilatory

activation of inorganic selenide to a selenophosphate[5] is followed by transfer of the selenium to a three-carbon intermediate at the level of transfer RNA to form selenocysteine[6].

Two kinds of evidence suggest that selenium's anticarcinogenic action may not involve its usual roles as an essential nutrient: (1) Se-dependent enzyme activities are already at a maximum at levels of selenium below its effective anticarcinogenic level; (2) forms of selenium that lack nutritional activity (unavailable for synthesis of Se-dependent enzymes) show good cancer preventing activity. If low molecular weight forms of selenium are involved in its anticarcinogenic activity, what are the forms and how are they produced? Scheme 1 summarizes known pathways of selenium metabolism that are discussed in regard to origins of chemopreventive activity.



Scheme 1. Selenium metabolism, emphasizing reactions for generating possible chemopreventive metabolites.

Biosynthesis of methylated selenium compounds. Methylation is an important pathway of selenium metabolism. Methylated selenoaminoacids are formed in plants[7]. Animals also synthesize methylated selenides, as summarized in Scheme 1 and reviewed elsewhere in more detail[7, 8]. Hydrogen selenide is the common intermediate in both the assimilatory pathway for synthesis of selenoproteins, and for the synthesis of methylated selenium excretory products. For inorganic selenite, reduction occurs by reaction with the major cellular thiol (glutathione) and certain dithiol proteins[9, 10, 11]. Hydrogen selenide also is formed through the action of a lyase on selenocysteine[12]. Selenomethionine can be converted to hydrogen selenide via selenocystathione and selenocysteine[13]. Methylation of the inorganic selenide by thiol methyltransferase[10, 14] forms methyl selenol and dimethyl selenide, and further methylation by thioether methyltransferase[15, 16] forms trimethylselenonium ion. These methyltransferases play a major role in sulfur, selenium, and tellurium metabolism.

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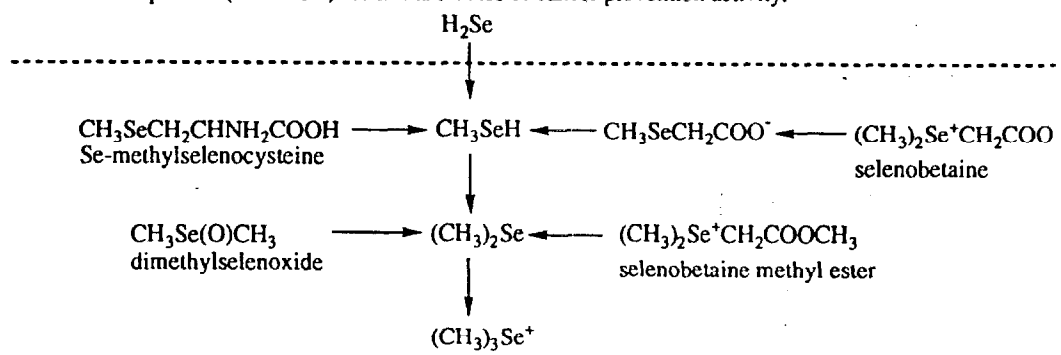
SELENIUM METABOLISM AND CHEMOPREVENTIVE ACTIVITY

Collaborative studies were begun with Dr. Clement Ip and Dr. Henry Thompson using animal models o mammary cancer to explore the basis for the anticarcinogenic action of selenium. We began our studies on the premise that (1) metabolites of sodium selenite were responsible for its anticarcinogenic activity, and (2) the quantitative output of such metabolites would increase as the dose of selenite was increased to the chemopreventive range. We sought compounds that would deliver chemopreventive activity but have low toxicity. Although there is considerable interest in the metabolites formed in the course of selenite metabolism via GSSeSG to hydrogen selenide, these also may be associated with toxic effects[17]. Methylation is the best known fate of selenium and the fully methylated metabolites are regarded as detoxified forms of selenium. Dr. Foster in this laboratory had shown that when animals were given methylated selenium compounds that enter t metabolic pathway beyond the inorganic pool, methylated metabolites were formed in large amounts[18, 19]. W chose such compounds (Scheme 2) for initial studies of cancer prevention activity.



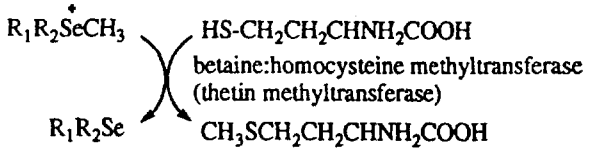
Scheme 2. Entry of methylated forms of selenium below the inorganic pool (indicated by dashed line).

The generation of a monomethylated form of selenium was a prominent feature of selenium compounds having good anticarcinogenic activity. Se-methylselenocysteine was about 650-fold more active than its sulfur analog[20], and a monomethylated form of selenium was the major excreted metabolite[21]. Its metabolism is discussed later in more detail. Selenobetaine and selenobetaine methyl ester had good anticarcinogenic activity[2] but dimethyl selenoxide and trimethylselenonium had little or no activity[22,24]. Even though half or more of the administered selenium was excreted as dimethyl selenide or trimethylselenonium ion with all four of these compounds, chemopreventive activity was markedly different, so that activity did not correlate with the excreti of the distal metabolites[21]. The metabolite profile also provided clear evidence that all of the methylated seleniu compounds underwent partial demethylation, so that even di- and tri-methylated precursors formed inorganic a mono-methylated products (the biochemical basis for demethylation is discussed below). The amount of inorganic selenium produced by demethylation of the active methylated selenium compounds was not correlate with their relative anticarcinogenic activity. Taken with other studies, these results indicate that formation of inorganic selenium is not essential for expression of anticarcinogenic activity, although it provides bioavailable

selenium for synthesis of selenoproteins. Clearly, the animal has extensive capabilities for interconverting these forms of selenium.

Demethylation of methylselenonium compounds

It was clear that demethylation of selenium occurred in animals, but the biochemical basis for such reactions had not been established. It was shown[23] that a homocysteine-dependent methyltransferase activity was present in liver that demethylates selenobetaines and trimethylselenonium:



When tested at near-optimal substrate concentrations, selenobetaine, selenobetaine methyl ester, and sulfobetaine gave much higher rates compared to betaine, the "physiological" substrate. Selenonium compounds were more active than their sulfonium analogues. Trimethylselenonium ion gave the highest rate of all the compounds tested. These results establish a biochemical basis for selenium demethylation, a metabolic process largely ignored in many discussions of selenium metabolism. This demethylation reaction probably competes with the production of sulfonium and selenonium derivatives by the recently discovered thioether methyltransferase[15,16], so that the steady-state level of such compounds in tissues that contain both enzymes (liver) will reflect the interplay of both enzyme activities. This is an important concept in view of the hypothesis that certain methylselenonium compounds generated by the thioether methyltransferase reaction may be mediators of selenium's anticarcinogenic action.

Anticarcinogenic activity and metabolism of Se-methylselenocysteine

One of the best chemopreventive forms of selenium in our studies was Se-methylselenocysteine[24]. It is a naturally-occurring form of selenium, and is a major constituent of plants grown on selenium-rich media[24]. This amino acid does not get incorporated into proteins, in contrast to selenomethionine, thus minimizing the possibility for excessive accumulation in tissues. As a monomethylated form of selenium, the metabolic point of entry is below the level of inorganic selenide. The metabolism of Se-methylselenocysteine, as described previously, gave monomethylated selenium as the major excretory metabolite. There was also extensive conversion to inorganic selenium, and this result was corroborated by the high bioavailability observed in other studies[20]. Because monomethylated selenium is the major excretory product, it seemed likely that direct scission of the Me-Se moiety from the amino acid would be catalyzed by an enzyme such as a lyase[19].

Cysteine conjugate β-lyase. Several pyridoxal phosphate-dependent enzymes that catalyze cleavage of the C-S bond of cysteine conjugates to form the thiol, pyruvic acid, and ammonia have been described, and have received considerable attention because of their importance to sulfur toxicology and metabolism[25]. The enzyme activity is predominantly located in liver and kidney, and in intestinal contents (almost all in association with

microorganisms). S-aryl-L-cysteine conjugates (having the sulfur attached directly to an aromatic ring) appear to be the best substrates for the tissue β-lyases of mammals as well as intestinal flora[25]. The microbial β-lyase has a broader substrate specificity and acts on S-alkyl as well as S-aryl-cysteine derivatives. Gut flora are exposed to high amounts of various cysteine conjugates present in diets, such as cysteine conjugates in kale or *Allium* species. It is apparent that intestinal flora may be involved in the metabolism of dietary Se-alkylselenocysteine derivatives. Recently it has been shown that cysteine conjugate β-lyase of kidney origin will cleave the C-Se bond to release alkyl- or aryl-selenols from alkyl- or aryl-selenocysteine derivatives[26]. Good activity was observed for the lower alkyl series of selenocysteine conjugates, whereas the coresponding sulfur analogues were inactive. For some selenocysteine conjugates, fairly rapid non-β-lyase scission was observed that may involve a β-elimination mechanism[27].

We propose that the thiols or selenols released by cysteine conjugate ß-lyase will be methylated by thiol methyltransfersase, and further methylated by the thioether methyltransferase to give the dimethylselenonium derivative[15, 16]. Se-glucuronidation also may occur, as observed with other organoselenium compounds[28]. Besides the action of cysteine-conjugate β-lyase on the selenocysteine conjugates, N-acetylation is likely to be a competing reaction, since this is a well-established activity for formation of mercapturic acids[29]. The relative activity of various Se-alkyl selenocysteine derivatives with respect to N-acetylation vs. scission to release the selenol may vary, and may be a factor to consider in designing anticarcinogenic forms of such compounds. Oxidation by monooxygenases to a selenoxide (see below) also may be a factor in regard to selenocysteine conjugate metabolism, favoring selenenic acid elimination[27].

Oxidation of selenoethers by microsomal monooxygenases

Dimethyl selenide is an excellent substrate for microsomal flavin monooxygenases, even at sub-micromolar concentrations[30]. The reaction is easily monitored by the oxidation of NADPH using purified pig liver enzyme. The selenoxide product undergoes rapid reduction back to the selenoether, and this facile redox cycling may be important in regard to some of the biological activities of selenium. A number of synthetic selenoethers also were shown to be oxidized to selenoxides[31]; cytochrome P_{450}-catalyzed oxidation was significant for some of the organoselenium compounds. Selenium analogs of sulfur aminoacids such as S-alkylcysteine derivatives and methionine that are substrates for certain flavin monoxygenase isozymes[32,33] also are likely to undergo oxidation *in vivo*. Because the selenoxide products undergo rapid reduction back to the selenoether, it is possible that those selenoethers that can undergo facile methylation will eventually be methylated to the selenonium derivative due to the sustained action of the thioether methyltransferase.

Anticarcinogenic activity and metabolism of selenocyanates

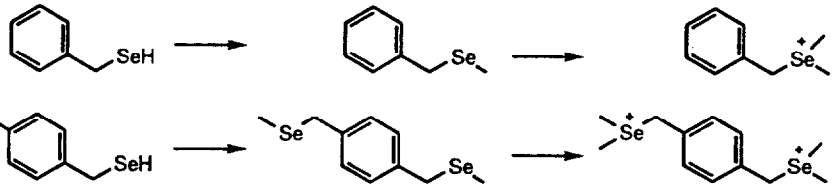
Benzylselenocyanate and various isomers of xylyl-bis(selenocyanate) were shown to be active in chemoprevention[34,35]. When fed to animals, the xylyl derivatives were relatively less toxic in relation to chemopreventive activity, due to a lower absorption from the intestinal tract[36]. A series of alkylselenocyanates evaluated for their ability to block the initiation phase (administered only at the time of carcinogen administration) showed increasing activity with increasing chain length up to five carbons[37]. The anticarcinogenic activity[38] and metabolism[39] of potassium selenocyanate also has been reported. In contrast to the relative inertness of

thiocyanate, which is excreted as an end product of sulfur metabolism in urine, potassium selenocyanate was efficiently catabolized and had similar bioavailability to other inorganic forms of selenium[40]. The cyanide moiety is converted to thiocyanate, as shown by labeling studies[39]. For organic selenocyanates, it is likely that scission of the Se-CN bond involves glutathione and is catalyzed by glutathione transferases, since the analogous organic thiocyanates are known to be substrates for this enzyme[41]:

$$RSeCN + GSH \longrightarrow RSeSG + HCN \quad (1)$$

$$RSeSG + GSH \longrightarrow RSeH + GSSG \quad (2)$$

The metabolism of benzyl selenocyanate to benzyl selenol and the disposition of the benzyl moiety has been described[42]. We suggest that further metabolism of the selenol intermediates formed from benzyl and xylylselenocyanates would occur by thioether methyltransferases, to give the mono- or bis- methyl selenides and dimethylselenonium derivatives:

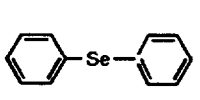


In a study of the metabolism of methylselenocyanate, about 40% of the dose was excreted as dimethyl selenide plus trimethylselenonium[43]; double-labeled studies showed retention of methyl by selenium in the products[43].

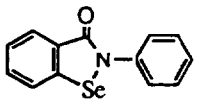
Anticarcinogenic activity and metabolism of phenyl selenides and triphenylselenonium chloride

The possible importance of lipophilic character for anticarcinogenic activity was suggested by the studies with benzyl or xylene-type selenocyanates, as well as aliphatic selenocyanates (RSeCN). A drawback of these types of selenocyanates is the facile scission of Se from the organic moiety. The bioavailability of methyl selenocyanate and benzyl selenocyanate is comparable to selenite, and 1,4-xylyl-bis(selenocyanate) also had substantial bioavailability, as measured by the restoration of glutathione peroxidase[38].

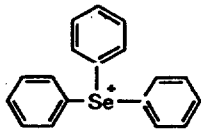
In order to retain lipophilic character but reduce the bioavailability of the Se, we turned to aromatic organoselenium compounds where Se is bonded directly to an unsubstituted benzene ring. Such compounds, and the phenyl selenide drug, Ebselen, have very low toxicity and bioavailability[44, 45]. These characteristics likely are explained by the inherent chemical and metabolic stability of the Se in such compounds, involving sp^2 bonding and delocalization of electrons of Se into the aromatic ring. Any biological activity of such compounds is more likely to be associated with the intrinsic molecule, rather than selenium released from the structure.



diphenyl selenide



Ebselen



triphenylselenonium

Of particular interest is the triphenylselenonium ion, since it has three benzene rings attached to selenium, but has a permanent positive charge due to the onium center, conferring solubility in water. Thus, triphenylselenonium ion is an amphiphilic or lipophilic cation, a class of compound having antitumor activity[46].

Triphenylselenonium (fed to animals as the chloride salt) proved to have very low toxicity and good efficacy at 10-30 parts/10^6 in the diet, giving the best ratio of efficacy to toxicity for any selenium compound tested to date[47]. Tissue selenium levels were increased only slightly by feeding a chemopreventive level of triphenylselenonium, in contrast to most forms of selenium used in chemoprevention[47]. This is a very favorable property, alongwith water solubility and lack of odor, for any agent being considered for use in cancer prevention. Very low toxicity but good cytostatic activity also was observed when triphenylselenonium chloride was added to cultured mammary tumor cells[48]. Cytostasis was associated with decreased cell proliferation and delayed cell cycle progression. Effects of triphenylselenonium on cellular metabolism (increased rate of glucose consumption and lactic acid production) were observed; this apparent enhancement of glycolytic metabolism may be a compensatory effect resulting from decreased mitochondrial energy production. Lipophilic cations are known to be accumulated in mitochondria because of the negatively charged mitochondria matrix, thus one possible site of action for triphenylselenonium chloride is mitochondria[48]. The activity of triarylselenonium compounds establishes a new class of chemoprevention compounds, and directs attention to anticarcinogenic selenium compounds having lipophilic character along with cationic properties, or the potential for generating such types of compounds when metabolized in animals.

Synthesis of [^{75}Se]triphenylselenonium derivatives. Little is known about the tissue distribution and metabolism of triphenylselenonium ion. To facilitate such studies, we have synthesized the radioactive compound by a series of reactions starting with commercially-available radioactive selenious acid. The method involves the classic sequence of converting the element to potassium selenocyanate, which is then reacted with diazotized aniline to form phenyl selenocyanate. Along with the selenocyanate, radioactivity was recovered in diphenyl selenide (relative yield of products 2:1, respectively). The structure of both products was confirmed by mass spectrometry. After converting the phenylselenocyanate to diphenyl selenide by reaction with phenyl lithium, the diphenyl selenide was converted to the dichloride and subjected to Friedel-Crafts reaction to form the triphenylselenonium product. This was adsorbed onto a weak-cation ion exchanger, and washed to remove impurities. Because of the dual retention mechanisms involvng hydrophobic interactions as well as electrostatic interactions, the triphenylselenonium remains bound to the ion exchanger during washing with 90% methanol (as well as 0.5 N perchloric acid), but is eluted by a combination of 50% methanol and 0.5 N perchloric acid, and crystallizes in this solvent in the cold as the perchlorate salt. Although the optimal conditions have not been worked out and the yield was low, the product was very pure. HPLC showed a single radioactive and ultraviolet peak having a spectrum and retention time (12.3 min) identical with that of standard triphenylselenonium chloride, using a perchlorate-perchloric acid eluting buffer (Figs. 1, 2). The UV maxima for triphenylselenonium perchlorate (266 and 272 nm) are at slighly lower wavelengths compared to triphenylsulfonium perchlorate (267 and 275 nm)[49], but otherwise the spectra are very similar. In a separate study comparing the triphenyl derivatives of the Group VI elements, the retention times increased in the order triphenylsulfonium (9.8 min), triphenylselenonium (11.4 min), triphenyltelluronium (17.2 min) using the polymer-based PRP-1 column. Using the same elution solvent with a C18 silica reversed phase column (TSK phenyl), the elution order was reversed.

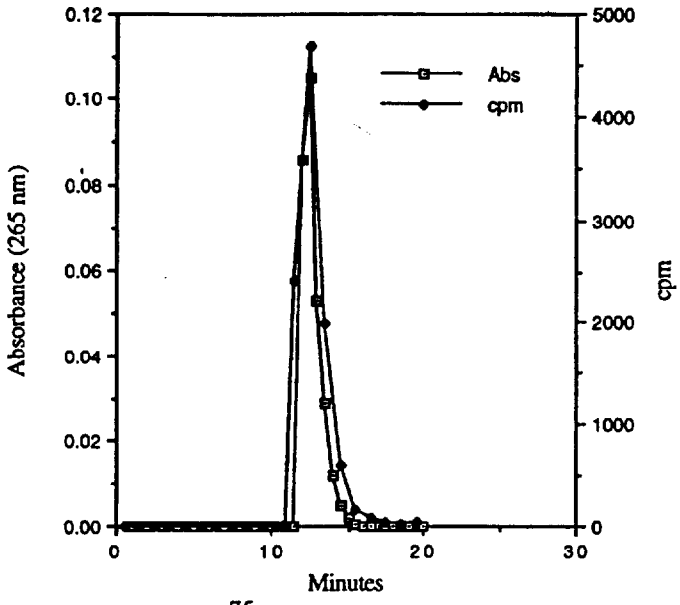


Fig. 1. Reversed phase HPLC of [^{75}Se]triphenylselenonium perchlorate. Sample: 40 μL [^{75}Se]$(C_6H_5)_3Se^+ClO_4^-$. Retention time: 12.3 min.

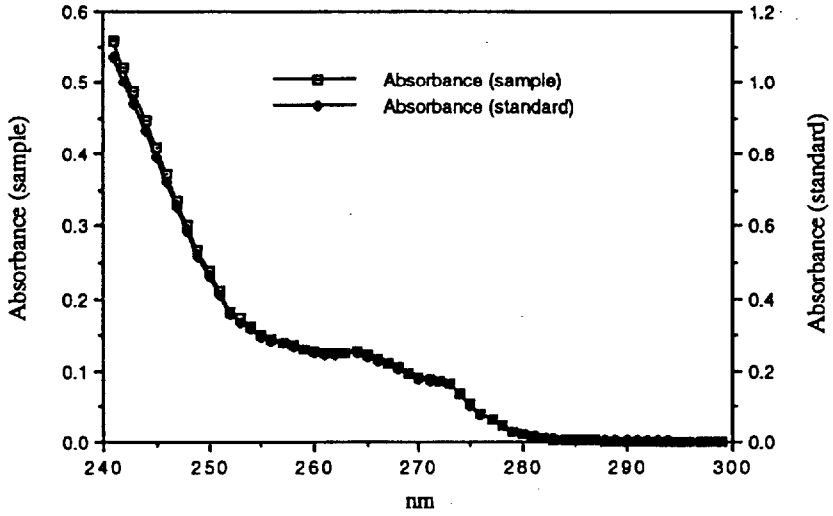


Fig. 2 HPLC diode array spectra of triphenylselenonium derivatives. Standard: 40 μL 1 mM $(C_6H_5)_3Se^+Cl^-$. Sample: 40 μL [^{75}Se]$(C_6H_5)_3Se^+ClO_4^-$. Retention times: 12.3 min.

DISCUSSION

Evidence has been summarized that supports a biosynthetic origin of active chemopreventive selenium metabolites involving the attachment of suitable carbon chains to a selenium atom (X):

$$X \longrightarrow R_1X \longrightarrow R_1R_2X \longrightarrow R_1R_2R_3X^+$$

The monomethylated form of selenium appears to be a critical metabolite formed by metabolism of inorganic selenium, or formed from precursors such as Se-methylselenocysteine. The monomethylated form appears to lack some of the adverse toxic effects associated with inorganic forms of selenium and hydrogen selenide (genotoxicity); one possible mechanism of action may be induction of apoptosis in cancer cells[50].

If alternative types of carbon chains (such as allyl) are available, more active metabolites might be formed. Plants such as *Allium* species can transfer allyl groups to sulfur, and possibly selenium. The C6 product formed by transfer of two allyl groups to sulfur can undergo methylation when metabolized in the animal to give a C7 onium product[16]. The point is that the potential activity of selenium can be enhanced in the course of being metabolized in plants, especially in those species that have specialized alkyl-group transfer capabilities. Furthermore, the higher chemopreventive activity of selenium compounds compared to sulfur analogs could involve superiority in *generating* the alkylated derivatives (greater nucleophilic character and greater availability of its electrons for alkylation, especially in forming the onium center). This factor would be in addition to any differences due to the elements once they are incorporated into a given chemical structure. The greater chemopreventive activity of garlic grown on selenium as compared to regular garlic has been demonstrated[51]. Thus, natural products formed from selenium in plants ultimately can give rise to more active chemopreventive metabolites in animals, as compared to the chemopreventive products formed in animals from inorganic selenium.

Triphenylselenonium chloride and related phenyl selenide derivatives represent novel organoselenium chemopreventive compounds with useful properties. They have greater metabolic stability because selenium is bonded directly to an unsubstituted benzene ring. Their mechanisms of action remain to be established.

ACKNOWLEDGEMENTS

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EXPERIMENTAL

Synthesis of [^{75}Se]triphenylselenonium chloride. The starting material was [^{75}Se]H_2SeO_3 obtained from the University of Missouri Research Reactor Facility, Columbia, Missouri. An aqueous solution containing 182 μCi of radioactivity plus 1 mmol (0.110 g) of carrier SeO_2 was treated with ascorbic acid to reduce the selenious acid to elemental Se. The aqueous phase was removed and the pellet of Se converted to KSeCN with 1 mmol KCN plus 1 drop of conc. ammonium hydroxide in 1 mL of water at 50°. Aniline (1 mmol) was diazotized by the slow addition of $NaNO_2$ to an aqueous HCl solution at 4°. The pH was adjusted to 4-5 by the addition of 1 M sodium acetate and the solution of KSeCN added to the chilled solution of diazotized aniline over a 30 min period. The organic products were extracted into dichloromethane and dried under nitrogen. The oil was taken

$(C_6H_5)_3Se^+Cl^-$.

up in 2:1 heptane:dichloromethane and chromatographed on a silica gel column using the same solvent. The first fraction collected yielded a colorless oil, identified as diphenylselenide by HPLC/UV diode array and mass spectroscopy. A second fraction (yellow, unidentified) was eluted followed by a third fraction identified as phenylselenocyanate on the basis of HPLC/diode array and mass spectroscopy. The phenylselenocyanate was reacted with phenyllithium in THF for 30 min at 0° under N_2, quenched with water, and extracted with dichloromethane, then purified by silica gel chromatography to give diphenyl selenide. The two diphenylselenide portions were combined and converted to the dichloride using nitric acid followed by HCl. The reaction mixture was then diluted with water and the suspended yellow solids extracted into chloroform and evaporated to dryness under nitrogen. A solution of the diphenylselenide dichloride in benzene was converted to triphenylselenonium chloride by the Friedel-Crafts reaction with excess $AlCl_3$ in five portions, at low temperature (about 8°). After 0.5 h a small piece of ice was added to the deep red solution, after which the triphenylselenonium chloride product (13 μCi, 7 % yield) was obtained by extraction with water.

Purification of [^{75}Se]triphenylselenonium by ion exchange chromatography. Adsorption onto a weak cation exchange column (Amberlite CG-50, H^+ form) followed by elution with aqueous methanol containing perchloric acid gave [^{75}Se]triphenylselenonium perchlorate, which crystallized as fine needles in the cold. Procedure: The aqueous solution of radioactive triphenyl selenonium chloride was adsorbed onto the column (previously washed with methanol and equilibrated with water). After sample application, the column was washed with water to remove a small amount of radioactive impurity, followed by aqueous methanol (to 90% methanol). After equilibrating the column with 50% aqueous methanol, elution was begun using 50% methanol containing 0.5 N perchloric acid. Fractions were collected and assayed for radioactivity. A broad peak containing 85% of the applied radioactivity was eluted, and these fractions were cooled to -20°. The crystalline product was collected and dissolved in a small volume of methanol for subsequent assay of purity by HPLC.

HPLC analysis. For analysis of triphenylselenonium chloride and related compounds, a polymer-based reversed phase column (Hamilton PRP-1, 1 x 10 cm, fitted with a guard column) was operated at 25°, using isocratic elution (1 mL/min) with methanol:water (65:35) containing 5 mM $NaClO_4$ plus 5 mM $HClO_4$, pH 2.5. A photodiode array detector (Waters model 991) was used to monitor the ultraviolet spectra of eluted compounds. For analysis of crystalline [^{75}Se]triphenylselenonium perchlorate, a fraction collector was used to collect 1 min fractions for direct assay of ^{75}Se by gamma ray scintillation counting.

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SHOULD SELENIUM ENRICHED VEGETABLES BE CONSUMED FOR THE PREVENTION OF CANCER ?

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Abstract

Selenium enriched broccoli, onions and garlic significantly reduce chemically induced mammary tumors in rats. Recent results with selenium supplementation in American subjects have shown significant reductions of certain cancers such as prostate, colon and lung. Se-methylselenocysteine is the major free selenocompound in selenium enriched onions, broccoli and garlic. This compound is most effective in reduction of chemically induced mammary tumors in rats. Results suggest that consumption of selenium enriched vegetables by humans will reduce the incidence of cancer. The present work investigates the most effective ways for enriching vegetables with selenium. In the initial experiment, applying selenium in a protected diffusion zone, there was a significant correlation between the amount of selenium added and the concentration of selenium in the broccoli florets. A concentration of 350 μg selenium per gram broccoli was obtained at the highest application rate of selenium. A subsequent experiment indicated that selenium was present at 5 to 6 times the concentration in florets from plants sprayed with selenium as compared to those where selenium was added to the soil. Greater foliar uptake of selenium occurs when a sticker is added to the aqueous solution. Spraying selenium three times at one week intervals with the third spraying shortly before the florets started to develop resulted in an accumulation of 450 μg selenium per gram. Spraying selenium on lettuce before maturity also resulted in greater deposition of this element as compared to application to the soil.

There have been almost two hundred trials conducted with laboratory animals of the effects of selenium on viral, chemical and spontaneously induced tumors, and the majority of them indicated a positive effect with this element (1). This took on additional significance when similar results were obtained with humans. Three human trials have been conducted on the effects of selenium on cancer and all of them have shown positive results. The first trial was conducted in China where the addition of selenium to table salt was shown to significantly reduce liver cancer (2). A second trial with humans was also conducted in China where the supplementation of selenium and vitamin E for seven years resulted in significant reductions in throat, stomach and colon cancers (3). This study was not considered definitive because it could not be determined whether selenium, vitamin E or the combination gave the response. The third trial was conducted in the United States with Americans who are considered to consume adequate amounts of dietary selenium (4). After supplementation with selenium as selenium enriched yeast (200 micrograms per day) for seven years, the incidence of colon, throat and lung cancers was reduced respectively by 60, 50 and 40%. Thus, super nutritional levels of selenium apparently give positive results even though nutritionally adequate levels of selenium are already consumed.

If high intakes of selenium are beneficial against certain cancers, then it is desirable to consider the optimum method for increasing the consumption of this element. Even though supplements are one avenue, another logical approach is to increase the selenium content in certain foods. This approach gives beneficial results in rats. Selenium enriched onions, garlic and Brazil nuts significantly reduced chemically induced mammary tumorsl (5).

Selenium enriched broccoli reduced the incidence of chemically induced mammary tumors as well (Ip, Buffalo, N. Y., personal communication). The present study investigates methods for enriching vegetables with selenium. Emphasis is on broccoli because this vegetable will take up high levels of selenium and also contains indole carbinol (6), chlorophyl (7) and sulforaphane (8); all of these compounds counteract tumors . Broccoli is a rich source of calcium, iron, and vitamins E and C. Preliminary results were also obtained with lettuce.

In the first experiment with broccoli, selenium was applied by the protected diffusion zone method (9). Levels of 4, 8 and 12 mg of selenium as sodium selenate were applied in the root zone . Selenium concentration in the broccoli florets was correlated with the quantity of selenium initially applied in the protected diffusion zone (figure 1). The highest level of selenium used resulted in about 280 micrograms selenium per gram broccoli.

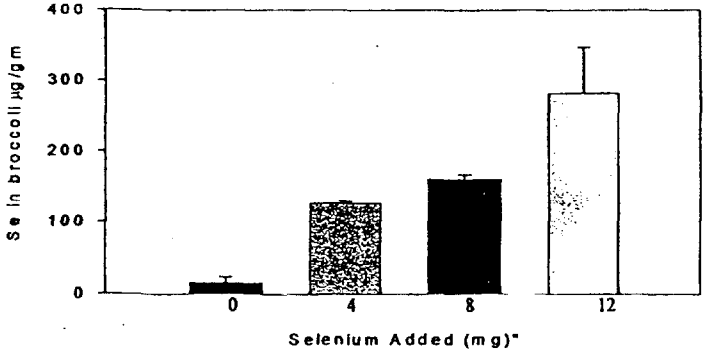

Figure 1. Uptake of selenium by broccoli using the protected diffusion zone method. The plants were grown in three liters of peat moss-perlite (1:1, vol) in the retaining pouch to which 0, 4, 8 and 12 mg selenium as sodium selenate was added. The florets were harvested 68 days after selenium application. Sprinter hybrid seed, Sakata Seed Co., Morgan Hill, CA, 95037, was used.. The values are means of three determinatons ± standard error.

Even though fairly high levels of selenium were obtained, it was reasoned that more efficient methods may be available to increase the content of selenium in broccoli florets. A comparison was made on the selenium content in florets when it was sprayed on the leaves versus addition to the soil in a garden plot. Concentrations in broccoli florets was about five times greater when selenium was sprayed on the leaves as compared to addition of the same amount of selenium to the soil (figure 2). In addition to higher concentrations in florets when selenium was sprayed on the leaves, this would result in less contamination of the environment such as the ground water as compared to addition to the soil.

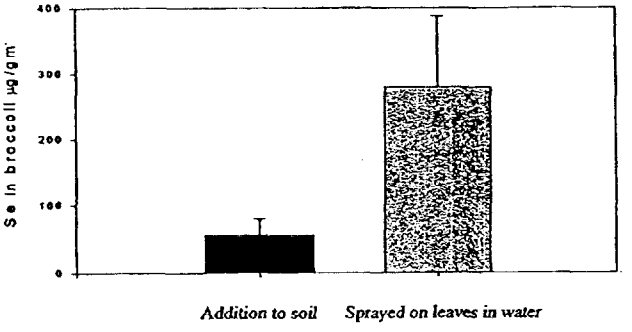

Figure 2. Uptake of selenium from soil versus leaves of broccoli. About 8 mg of selenium as selenate were sprayed on leaves of each plant or placed at the roots of each plant. The selenium was added or applied about 3 weeks before the florets started to develop. The broccoli was grown in black clay soil in a garden plot. Waltham 29 broccoli seed from Ed Hume Seeds, Inc., P. O. Box 1450, Kent, WA, was used. The values are the means of three determinations ± standard error.

The effects of sticker and the number of spray applications on the uptake of selenium by florets of broccoli were studied. When a sticker was included almost three times as much selenium was taken up by the florets as compared to just water alone (table 1). Also, as the number of sprayings was increased the concentration of selenium in the florets increased as well. Therefore, a sticker will increase the transport of selenium from the leaves to the florets.

Table 1. Effects of Sticker on the Uptake of Selenium by Broccoli

Treatments	Concentration of Se (µg/gm)
One spray only in water	41±27
One spray with water and sticker	114±83
Two sprays with water and sticker	232±50
Three sprays with water and sticker	449±270

Values are means of 3-5 determinations ± standard errors. The sticker used was sta-stuk "m", The Chas H. L. Lilly Co. Portland, OR. Waltham 29 broccoli seed was used.

In our studies on the uptake of selenium by florets, it was found that the species of broccoli greatly influenced the uptake of this element when sprayed on the leaves. About 450 micrograms of selenium per gram floret was obtained when selenium was sprayed on leaves of Walthan 29, but much less was obtained with Hybrid Packman (figure 3). Only 60 to 70 micrograms selenium per gram floret were obtained with the hybrid variety. Therefore, the species of broccoli can have a very marked effect on the uptake of selenium and thus must be considered in these types of studies.

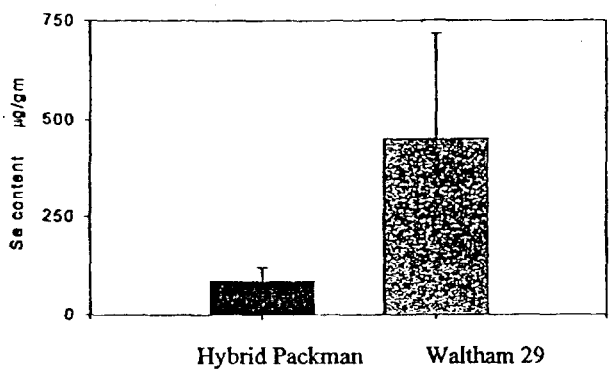

Figure 3. Effect of species of broccoli on uptake of selenium. Approximately 8 mg selenium as selenate was sprayed on the leaves of broccoli three times at one week intervals with the last sprayings just before the florets started to develop. The Hybrid Packman seed was obtained from Gurney's Nursery, Yankton, S. D., 57079. The plants were grown in black clay soil. The values are means of three determinations ± standard error.

In addition to the broccoli florets, interest has been generated with broccoli sprouts. For example, sulforaphane, a compound with anticarcinogenic activity, was found to be present at 8 to 10 times greater in broccoli sprouts as compared to broccoli florets (10). When broccoli seeds were sprouted and grown in various concentrations of selenium, a linear uptake was obtained with concentration levels up to 20 micrograms selenium per ml (figure 4). Increasing the selenium concentration to 30 micrograms per ml did not appear to be an advantage because less uptake was obtained with higher levels as compared to 20 micrograms per ml. A level of 40 micrograms selenium per ml was toxic to the seed. The seeds sprouted a little but did not grow with this level of selenium.

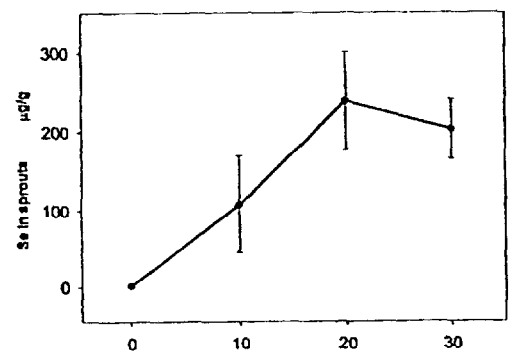

Figure 4. Selenium uptake by broccoli sprouts. Packman broccoli seeds were soaked in aqueous solutions of selenium at various concentrations for three hours. The seed were Packman Hybrid from Nichols Garden Nursery, Albany, OR. After the solutions were decanted, the seeds were germinated at 30 C for 10 days. The values are the means of three determinations ± standard error.

Broccoli sprouts purchased from a commercial source were incubated either with 20 or 30 micrograms selenium per ml. There was very little uptake by 24 hours of incubation, but significant uptake occurred at 48 hours of incubation (figure 5), and since this was the last point it is not known whether longer incubation time would be required

to reach a plateau. However, the broccoli sprouts were starting to turn brown by this time.

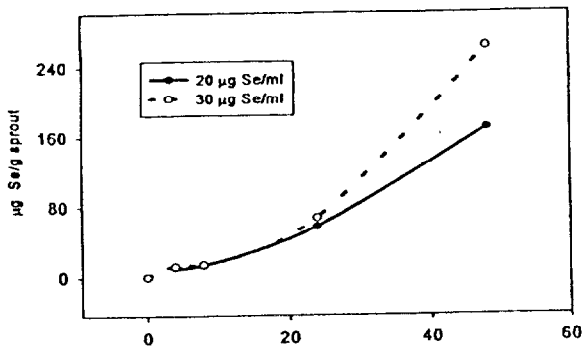

Figure 5. Broccoli sprouts were purchased from a commercial source (Cub Foods, Corvallis, OR) and incubated at room temperature with either 20 or 30 μg selenium per ml for various times. Each value is a single determination.

A comparison was made on the uptake of selenium by broccoli, clover, alfalfa and bean sprouts which were purchased from a commercial source (figure 6). The greatest amount of uptake occurred with broccoli and alfalfa sprouts and the least amount was taken up by clover sprouts. The amount taken up by beans sprouts was intermediate between clover and broccoli sprouts.

Bunch lettuce was investigated in a preliminary study, and similar results were obtained to broccoli with application of selenium to the soil versus the leaves (figure 7). Over 13 times as much selenium was present in lettuce (after washing) when it was sprayed on the leaves as compared to application to the soil. About 22 percent of the selenium on the leaves was removed when the lettuce was washed, suggesting that the remainder was incorporated into the leaves. Therefore, this preliminary work suggests that lettuce can be easily enriched with selenium.

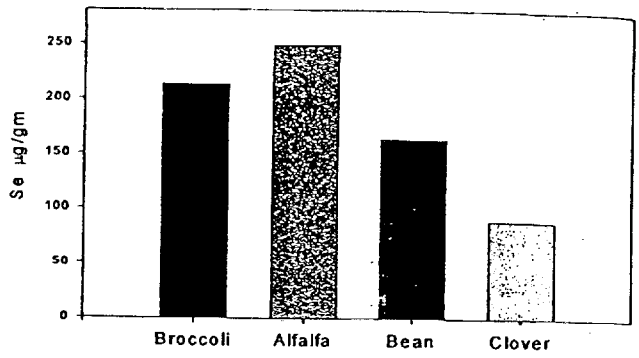

Figure 6. Broccoli, alfalfa, clover and bean sprouts were purchased from a commerical source (Cub Foods, Corvallis, OR) and incubated at room temperature with 30 μg selenium as selenate for 24 hours. Each value is a single determination.

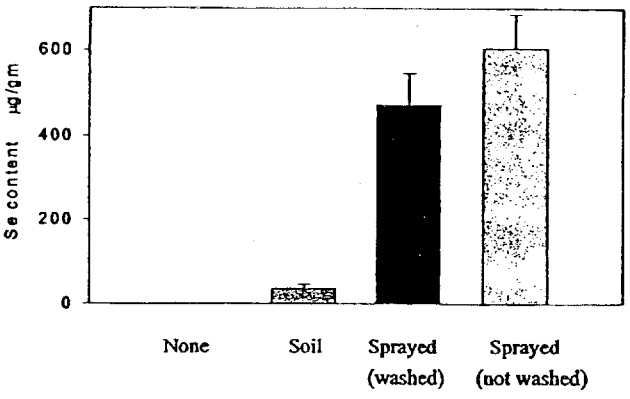

Figure 7. Uptake by lettuce of selenium either sprayed on the plant leaves or added to the soil. The seed was (Buttercrunch), purchased from Nichols Garden Supply, Albany, OR., and was grown in sandy loam soil. About 10 mg of selenium as selenate was either added to the soil or sprayed on the leaves two times at one week intervals. The last treatment was two weeks before the leaves were harvested. The values are means of three determinations ± standard error.

A positive correlation appears to exist between the effectiveness of selenium enriched vegetables as anticarcinogenic agents and the Se-methylselenocysteine content. Selenium enriched garlic is the most effective against tumorigenesis, followed by enriched broccoli and onions in decreasing order (5) against tumorigenesis, and the Se-methylselenocysteine content follows the same pattern (11). It will be interesting to determine whether this pattern holds true with other selenium enriched vegetables. If it does, then the determination of the Se-methylselenocysteine content of enriched plants could be used to predict their effectiveness as anticarcinogenic agents.

Acknowledgment

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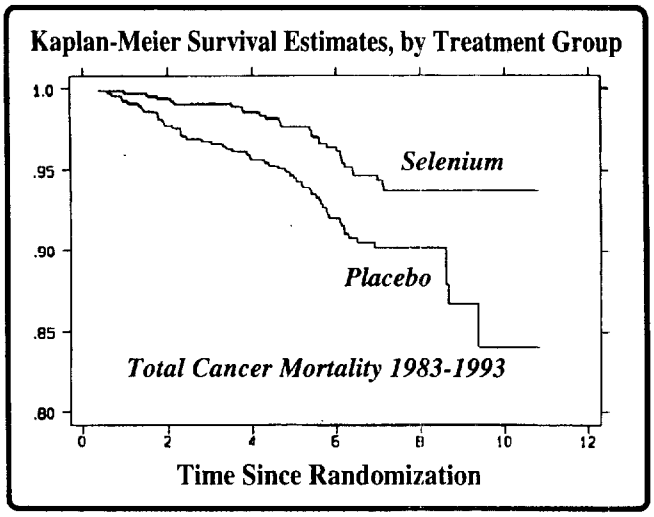
NOVEMBER 1997

Meet the Author at STDA SYMPOSIUM 10-13 MAY 1998 SCOTTSDALE (ARIZONA) Last Call For Papers Page 4

Recent Developments in the Prevention of Human Cancer with Selenium

Larry C. Clark*

Background: On December 25 1996, Dr. Clark and colleagues published results of the first randomized clinical trial in a western population that observed a reduced incidence of cancer resulting from the use of a selenium supplement. This trial builds on years of experimental and epidemiologic research by numerous investigators into the health effects of the essential trace element selenium. Dr. Clark and colleagues from both Europe and America are actively continuing their research and developing new projects to determine the health benefits of selenium supplementation. These collaborations include the development of a general population cancer prevention trial in over 50,000 subjects with several dosages of selenium in six European nations and America.



The publication of the results from the "Nutritional Prevention of Cancer (NPC) Trial with Selenium" in the Journal of the American Medical Association has caused a surge in interest in the health effects of selenium. This study is the first double blind cancer prevention trial in a western population to report that a nutritional supplement can reduce the risk of cancer. In June, Dr. Clark presented the results of the trial for prostate cancer at the conference on "Dietary Micronutrients and Human Cancer Risk" in Aarhus, Denmark. The possibility of preventing prostate cancer with Se supplementation created considerable interest at the conference since Denmark, Finland, Norway and Sweden have some of the highest mortality rates from prostate cancer in Europe. A follow up meeting was held at the Danish Cancer Society campus in Copenhagen to discuss what would be required to replicate the results of the NPC trial with a general population cancer prevention trial in northern Europe. As a result of this meeting, a workshop was held in September 1997, that invited scientists and epidemiologists from six European nations and America to discuss the feasibility of designing and conducting a cancer prevention trial with Se. This workshop was an outstanding success and plans for developing and implementing the project "Selenium for Cancer Chemoprevention in Europe and America: A Randomized Clinical Trial" continue at a rapid pace.

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The Nutritional Prevention of Cancer with Selenium

The origin of the increasing interest in the use of Se for cancer prevention is the publication of the results of our cancer prevention trial, "The Nutritional Prevention of Cancer with Selenium." This trial observed that patients assigned to take 200 mcg of Se per day had a 37% lower incidence of cancer and 50% fewer cancer deaths during the first decade of observation. This study is a double blind randomized cancer prevention trial. The first patients were enrolled on September 15 1983, and randomized to the treatment of 200 mcg of Se per day from a high Se yeast (Selenomax, produced by Nutrition 21, La Jolla, CA) or to a placebo of Brewer's yeast. This study was a double blind clinical trial in which neither the patient nor their physicians knew which treatment the patient had been assigned to. Patients in this trial were treated and followed in a double blind manner until February of 1996. At that time, all patients and investigators were informed of the results of the trial, and the treatment group to which they had been assigned. The early unblinding and reporting of the trial was recommended by the trial's safety monitoring committee after a thorough discussion of the trial results.

The decision to report the results of the trial early was based primarily on the observed 50% decrease in cancer mortality. Additional consideration included the consistency of the trial results for the three leading sites of cancer (lung, prostate and colorectal) and the lack of effect on the primary study endpoints of skin cancers. Compared to the placebo group, the incidence of cancer in the Se treatment group was 46% lower for lung cancer, 48% lower for colorectal cancer and 63% lower for prostate cancer. There was no significant treatment effect for skin cancers, although the incidence of basal cell carcinoma of the skin was 10% higher and the incidence of squamous cell carcinoma of the skin was 14% higher in the treatment group. The consistency of the treatment effect was apparent in the study clinics with six of the seven clinics having lower rates of total cancer incidence and mortality in the treatment group. In addition, total mortality was lower in the treatment group for 9 out of the 10 years of the study, while total cancer incidence and mortality was lower in the treatment group for 8 out of 10 years. Another important aspect of the trial was its ability to ascertain the safety of long term supplementation with selenium at the 200 mcg per day dose.

At the time the trial was unblinded to patients in February of 1996, all patients in both the treatment and placebo groups were invited to take 200 mcg of Se/day until December of 1998, which is the planned end of the intervention phase of the trial. We continue to contact patients semi-annually to ascertain new health events and provide them with an additional supply of pills.

Selenium Supplementation Reduces the Incidence of Prostate Cancer

At the conference on "Micronutrients and Human Cancer Risk", Dr. Clark presented the effect of selenium supplementation on the incidence of prostate cancer in the NPC trial. During the first ten years of follow up there were 35 new cases in the placebo group, but only 13 cases in the selenium treatment group. This is a 63% reduction in the incidence of prostate cancer! If you assumed that the maximum effect of selenium supplementation on prostate cancer incidence requires at least two years of supplementation then the treatment effect increased to a 74% reduction in incidence. Using information on each patient's pre-randomization plasma selenium level, those patients who were in the lowest third of plasma selenium levels had over a 90% reduction in incidence. There was only one case in the treatment group compared to 13 cases in the placebo group. For the middle third, there were 4 cases in the treatment group and 13 in the placebo group, a 70% reduction in incidence. There was only a 15% reduction in incidence in the highest third of plasma selenium levels, but this was based on 8 cases in the treatment group versus 9 in the placebo group. Interestingly, there was also a strong suggestion of an enhanced treatment effect for men under age 65 compared to older men, a 91% reduction in incidence versus a 51% reduction. The consistency and biologic plausibility of these results strongly suggest that selenium supplementation can reduce the incidence of prostate cancer. However, before these results are fully accepted by the medical and scientific communities and before public health recommendations can be made, the results of this important trial need to be replicated in the general population.

Selenium for the Chemoprevention of Cancer in Europe and America: A Randomized Controlled Trial (RCT)

The workshop in Copenhagen plan a cancer prevention trial in Europe with selenium held on September 18-20, 1997, at the campus of the Danish Cancer Society. It was a very successful meeting as group quickly reached the decision that it was of paramount importance that a major cancer prevention trial be conducted to replicate the results of the NPC trial. If this trial observed a significant reduction in cancer incidence with selenium supplementation, it would have major public health consequences for the low selenium regions of Northern Europe and America. The remainder of the workshop was spent developing the protocol for the trial.

To briefly summarize the results of the workshop, it was decided that the trial would have 7 cohorts, one in each of the participating countries. Currently, Denmark, Finland, Norway, the Netherlands, Sweden, the United Kingdom and America are planning to participate. Each cohort would recruit and randomize approximately 7,500 subjects, with the goal of recruiting approximately equal numbers of men and women between the ages of 60-74. The subjects would be randomized to receive either placebo or one of three dosages of selenium, 100 mcg/d, 200 mcg/d or 300 mcg/d. The rationale for the selection of the dosages for the trial was that the 200 mcg dose would provide a replication of the NPC trial while 100 mcg/d and 300 mcg/d would

investigate if a smaller dose was as good as the 200 mcg/d dose or if a larger dose was more effective. The use of three dosages also has an advantage in that 75% of patients will be receiving a selenium supplement. With approximately 52,500 subjects randomized, the trial will have the statistical power to detect at least a 10% reduction in total cancer incidence with 90% power.

Another major strength of this proposed trial is that there are several available options for improving the selenium intake of populations. First is the addition of selenium to fertilizer to improve the concentration of selenium of crops and livestock. Finland has adopted this approach and has demonstrated its effectiveness in enhancing the selenium status of its population. This approach could be rapidly adopted by countries with low selenium regions to enhance selenium status, although there would likely be considerable debate regarding the appropriate level of selenium in fertilizers and what should be the target concentrations in crops and livestock. The second approach would be the fortification of food with selenium. This is already done with some baby food formulas and could be extended to additional foods. The issues raised by these two approaches require the involvement of the national ministries and agencies that would have responsibility for these approaches. The steering committee for the trial intends to engage appropriate agencies in each country in a dialogue that would facilitate their decision making regarding the enhancement of the selenium status of their populations. The final approach for increasing selenium status is through the use of selenium supplements. This approach allows individuals who have particular health concerns, such as being at high risk of cancer, to supplement themselves while the other population based approaches are initiated on regional and national levels. The integration of these three approaches should allow for a relatively rapid public health initiative that could begin to lower cancer incidence rates in entire populations at risk of cancer because of sub-optimal selenium levels.

The potential impact of this trial strongly argues for its rapid funding and implementation. A combination of private individuals and foundations, as well as business and government sources are being approached to help finance the estimated $30 million budget of the six year project. The Steering Committee for the project meets again on January 17th and 18th, 1998, in Copenhagen to finalize the study protocol and the data collection forms. This would allow the first vanguard cohort studies to begin in early 1998 or when funding for the project becomes available.

Book Review...

Selenium in Food and Health

By Conor Reilly

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ORGANICS

Print Date: 8/20/98

Material Safety Data Sheet

Se-methylseleno-L-cysteine

Section 1 - Chemical Product and Company Identification

MSDS Name:
Se-methylseleno-L-cysteine

Catalog Numbers:
30064-0000, 30064-2500

Synonyms:

Company Identification:
Acros Organics N.V.
Janssen Pharmaceuticalaan 3a
2440 Geel
Belgium,

Company Phone Number:
0032(0)14575211

Emergency Phone Number:
0032(0)14575299

CHEMTREC Phone Number, US:
(800) 424-9300

CHEMTREC Phone Number, Europe:
(202) 483-7616

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name:	Percent	EINECS/ELINCS
26046-90-2	Se-methylseleno-L-cysteine	100.0	Not available.

Hazard Symbols:
Risk Phrases:

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: No information available.
Caution! May cause eye and skin irritation. May cause respiratory and digestive tract irritation. The toxicological properties of this material have not been fully investigated.
Target Organs: none known.

Potential Health Effects

Eye:
Dust may cause mechanical irritation.

Skin:
No information regarding skin irritation and other potential effects was found.

Ingestion:
May cause irritation of the digestive tract. The toxicological properties of this substance have not been fully investigated.

Material Safety Data Sheet

Se-methylseleno-L-cysteine

Inhalation:
Inhalation of dust may cause respiratory tract irritation.

Chronic:
No information found.

Section 4 - First Aid Measures

Eyes:
Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Get medical aid immediately.

Skin:
Get medical aid. Flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.

Ingestion:
If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Inhalation:
Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

Notes to Physician:
Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information:
As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.

Extinguishing Media:
Use agent most appropriate to extinguish fire.

Autoignition Temperature:
No information available.

Flash Point:
No information available.

NFPA Rating:
(estimated) Health: 1; Flammability: 0; Reactivity: 0

Explosion Limits:
Lower: Upper:

Section 6 - Accidental Release Measures

General Information:
Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks:
Clean up spills immediately, observing precautions in the Protective Equipment section. Sweep up or absorb material, then place into a suitable clean, dry, closed container for disposal. Avoid generating dusty conditions. Provide ventilation.

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Se-methylseleno-L-cysteine

Section 7 - Handling and Storage

Handling:
Wash thoroughly after handling. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation.

Storage:
Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances.

Storage Code:
Gray

Section 8 - Exposure Controls, Personal Protection

Engineering Controls:
Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name:	ACGIH	NIOSH	OSHA
Se-methylseleno-L-cysteine	None listed.	None listed.	None listed.

OSHA Vacated PELs

Personal Protective Equipment

Eyes:
Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin:
Wear appropriate protective gloves to prevent skin exposure.

Clothing:
Wear appropriate protective clothing to prevent skin exposure.

Respirators:
Follow the OSHA respirator regulations found in 29CFR 1910.134 or European Standard EN 149. Always use a NIOSH or European Standard EN 149 approved respirator when necessary.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Color: No information available.
Odor: No information available.
pH: No information available.
Vapor Pressure: No information available.
Vapor Density: No information available.
Evaporation Rate: No information available.
Viscosity: No information available.
Boiling Point: No information available.
Freezing/Melting Point: No information available.
Decomposition Temperature: No information available.
Solubility: No information available.

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Se-methylseleno-L-cysteine

Specific Gravity/Density: No information available.
Molecular Formula: C4H9NO2Se
Molecular Weight 182.0285

Section 10 - Stability and Reactivity

Chemical Stability:
Stability unknown.

Conditions to Avoid:
Incompatible materials, dust generation.

Incompatibilities with Other Materials
Oxidizing agents.

Hazardous Decomposition Products
Carbon monoxide, oxides of nitrogen, carbon dioxide.

Hazardous Polymerization
Has not been reported

Section 11 - Toxicological Information

RTECS:
CAS# 26046-90-2 unlisted.

LD50/LC50:
CAS# 26046-90-2:
No information available.

Carcinogenicity:
CAS# 26046-90-2: Not listed as a carcinogen by ACGIH, IARC, NIOSH, NTP, OSHA, or CA Prop 65.

Epidemiology:
No information available.

Teratogenicity:
No information available.

Reproductive:
No information available.

Mutagenicity
No information available.

Neurotoxicity
No information available.

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Dispose of in a manner consistent with federal, state, and local regulations.

RCRA D-Maximum Concentration of Contaminants
None of the components are on this list.

RCRA D Series - Chronic Toxicity Reference Levels
None of the components are on this list.

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RCRA F Series Wastes
None of the components are on this list.

RCRA P Series Wastes
None of the components are on this list.

RCRA U Series Wastes
None of the components are on this list.

RCRA Substances Banned from Land Disposal
None of the components are on this list.

Section 14 - Transport Information

	US DOT	IATA	IMO	RID/ADR	Canadian TDG
Shipping Name:	No information available.	No information available.	No information available.	No information available.	CHLOROTOLUENES
Hazard Class:					3
UN Number:					UN2238
Packing Group:					III
Additional Info:					FLASHPOINT 47 C

Section 15 - Regulatory Information

US Federal

TSCA
CAS# 26046-90-2 is not listed on the TSCA Inventory. It is for research and development use only.

Health and Safety Reporting List
None of the components are on this list.

Chemical Test Rules
None of the components are on this list.

TSCA Section 12b
None of the components are on this list.

TSCA Significant New Use Rule (SNUR)
None of the components are on this list.

CERCLA Reportable Quantities (RQ)
None of the components are on this list.

SARA Threshold Planning Quantities (TPQ)
None of the components are on this list.

SARA Hazard Categories
None of the components are on this list.

SARA Section 313
None of the components are on this list.

Clean Air Act - Hazardous Air Pollutants (HAPs)
None of the components are on this list.

Clean Air Act - Class 1 Ozone Depletors
None of the components are on this list.

Clean Air Act - Class 2 Ozone Depletors
None of the components are on this list.

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Clean Water Act - Hazardous Substances
None of the components are on this list.

Clean Water Act - Priority Pollutants
None of the components are on this list.

Clean Water Act - Toxic Pollutants
None of the components are on this list.

OSHA - Highly Hazardous
None of the components are on this list.

US State

State Right to Know

California Prop 65

California No Significant Risk Level
No information available.

European/International Regulations

European Labelling in Accordance with EC Directives:
Hazard Symbols:
Risk Phrases:
Safety Phrases: S 28A After contact with skin, wash immediately with plenty of water.
S 37 Wear suitable gloves.
S 45 In case of accident of if you feel unwell, seek medical advice immediately (show the label where possible).

WGK (Water Danger/Protection)
No information available.

Canadian DSL/NDSL
None of the chemicals in this product are listed on the DSL/NDSL list.

Canadian WHMIS Classifications
This product has a WHMIS classification of D2B.

Canada Ingredient Disclosure List
CAS# 26046-90-2 is not listed on Canada's Ingredient Disclosure List.

Exposure Limits

Section 16 - Other Information

Color Information has been
MSDS Creation Date: August 20, 1998
Revision Date: Original.

Essentiality and Toxicity of Selenium in Humans

Se ug/d	ug/kg BW	Chemical Form	Effects
<11	<0.20	Dietary	Keshan's Disease Keshan Beck's Disease
16	0.31	Dietary	Minimum Dietary Requirement
41	0.67	Dietary-75% Se-Met	Adequate Dietary Requirement
55	-	Dietary	RDA for Women
70	-	Dietary	RDA for Men
400	-	Dietary	Suggested Maximum Safe Dietary Limit
600	11	Dietary	Individual Maximum Safe Limit
819	15	Dietary	Maximum Safe Limit (NOAEL)
900	17	Dietary	Low Level Toxicity (individual LOAEL)
1000	-	$Na_2 Se 0_3$	Personally known intake for years
1540	28	Dietary	Low Level Toxicity (mean LOAEL)
1,600	30	Dietary	Adverse Effective Level
5,000	90	Dietary	Selenosis, hair and nail loss
15,000	270	Dietary	Overt Selenosis